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PHOTORESPONSE OF BIMOLECULAR LIPID MEMBRANES PIGMENTED WITH RETINAL AND VITAMIN A ACID

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SUMMARY

Measurements of the time response of photovoltages and of photocurrents are reported which were made in lipid bilayers pigmented with retinal and vitamin A acid, respectively. To exclude artifact photosignals due to photoreactions taking place in the redox agent ($K_3[Fe(CN)_6]$) adjacent to one side of the bilayer, the membranes were illuminated with monochromatic light in the absorption maximum of vitamin A. The shape of the photosignals was found to depend very distinctly on the pigment used in the bilayer. No biphasic photosignals were measured in retinal–lipid membranes whereas the photoresponse in vitamin A acid–lipid membranes was pronouncedly biphasic and strongly dependent on externally applied d.c. membrane potentials. The change of shape of the photosignals in retinal–lipid membranes in the course of an experiment showed that retinal was oxidized to vitamin A acid. A photochemical reaction is proposed which takes account of the oxidation and of the measured time response of the photosignals. Two different electrical analogy models were used which simulate quantitatively the photoresponse of retinal–lipid and of vitamin A acid–lipid membranes.

INTRODUCTION

In recent years a number of articles have been published on photoeffects in pigmented lipid bilayer membranes. The aim of the studies was to correlate the photo processes that occur in natural membranes to those that can be produced in pigmented model membranes. Most of the investigations were aimed at a better understanding of the plant photosynthesis[1–7] and of the visual process[8] in retinal rods[9–11]. The early receptor potential developing in the retina[12] consists of two voltage components of opposite sign. It seems to be the primary electrical signal in the visual process. Recently, Kobamoto and Tien[9] measured similar biphasic potentials in lipid bilayers doped with all-*trans* retinal when one side of the membrane was in contact with a redox system. The occurrence of biphasic photo-potentials was also reported by Ullrich and Kuhn[11]. The system bilayer–electrolyte they used was made unsymmetrical by adsorbing dye molecules to one side of the membrane only.

The aim of the experiments reported here is to describe quantitatively the time response of the photoelectric signals generated in lipid bilayer membranes

pigmented with all-*trans* vitamin A aldehyde (retinal) and all-*trans* vitamin A acid, respectively. In order to establish an unsymmetrical charge distribution across the bilayer upon illumination a redox agent was added to one side of the electrolyte solution contacting the membrane. It could be shown that the time response of the photosignals generated by retinal membranes was distinctly different from the photoresponse of bilayers pigmented with vitamin A acid molecules. Consequently different photoreactions will have to be used to describe the experiments. Electrical equivalent circuits consisting of linear elements will be proposed to simulate the measured time response of the photosignals. These analogy models will be compared with the models based on the photochemical processes leading to the photoelectrical signals in the pigmented membranes.

METHODS

Fig. 1 shows a schematic drawing of the experimental set-up. A teflon cell was used containing two compartments with a hole of 2-mm diameter in the separating wall across which the lipid bilayer is spread[13,14]. Both compartments are filled with an electrolyte solution. The outer wall of compartment 2 is sealed with a quartz window to allow short-wavelength light to pass from the large aperture Bausch and Lomb monochromator M. As a light source L either a 250 Watts tungsten halogen lamp or a 75 Watts high pressure mercury arc is used. The formation of the bilayer is observed through the glass window in the outer wall of compartment 1. The cell is mounted in a thermostat and the temperature of the membrane monitored by a sealed platinum resistor in the electrolyte of compartment 2. The temperature is set at 22 °C.

Rectangular light pulses with a rise time < 5 ms are let through the shutter S (Fig. 1). The open-loop photovoltage measurements are made with a Keithley vibratin greed electrometer, model 640 (amplifier A in Fig. 1), with an input impedance $R > 10^{16} \Omega$ and a rise time < 200 ms. The amplifier A is replaced in the photocurrent measurements by a Keithley high-speed picoammeter, model 417. The ammeter is operated with an input impedance R of $10^7\ \Omega$ and a rise time < 20 ms (the actual band-width of the instrument had to be reduced to improve the signal/noise ratio).

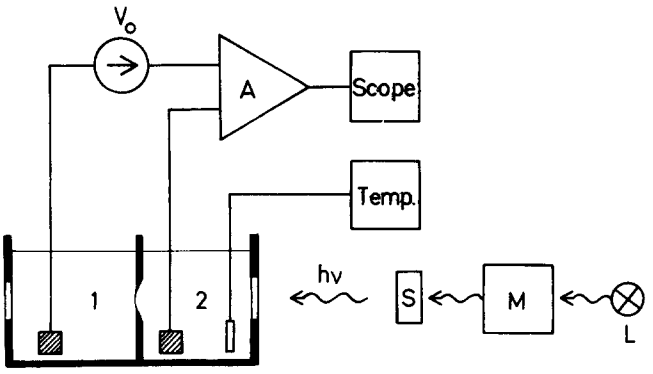


Fig. 1. Schematic drawing of the experimental set-up. V_0, voltage source; A, measuring amplifier; S, light shutter; M, monochromator; L, light source. The external voltage source V_0 is parallel to the amplifier A when measuring open-loop photovoltages *versus* V_0 (four-point probe set-up).

The output of the amplifier A is recorded either on a storage scope or on an X-Y-recorder. Spurious electrode potentials across the membrane which generate offset currents can be compensated with a d.c. voltage source V_0 (Fig. 1) whose polarity can be chosen. The same source is used to apply d.c. offset potentials across the membrane. Two separate electrodes are used to measure the potential across the membrane in those experiments where an offset d.c. potential V_0 is applied. This four-point probe set-up eliminates the voltage dividing effect otherwise occurring across the membrane resistance and the high input impedance of the voltmeter. The electrical signals are measured with reversible Ag–AgCl electrodes carefully shielded from direct light to minimize the generation of light-induced electrode potentials[15].

The lipid stock solution is prepared by dissolving 25 mg of synthetic dioleoyl-L-α-lecithin (Supelco) in 2.5 ml of *n*-decane (Merck). The membrane solution is always made just prior to the experiments. 1 mg of retinal is dissolved in 100 μl of lipid stock solution to which 80 μl of *n*-decane and 20 μl of ethanol (Merck) are added. The retinal as well as the vitamin A acid was synthesized at Roche (98% pure, 2% all-*trans* vitamin A polymer products). The membrane solution is kept for no more than 4 h. Great care needs to be taken to avoid exposure of retinal or of the membrane solution to light and air. All preparations have to be performed in the dark in an inert gas atmosphere.

Both compartments of the cell (Fig. 1) are filled with an electrolyte solution of 0.1 M KCl. Before the cell is filled spectroscopic grade argon is bubbled through the solution for 20 min in order to eliminate oxygen and carbon dioxide. After the membrane becomes black[14], $K_3[Fe(CN)_6]$ is added to compartment 1 ($2 \cdot 10^{-3}$ M). This redox system proves to be very reliable and produces only spurious photo-signals of its own at the wavelength where the photo-excitation of retinal is a maximum. This coincidence improves the signal/noise ratio of the measurements considerably. It makes it also easy to distinguish between artifacts and the "real" photo-signals (see below). Furthermore, $Fe^{III}(CN)_6^{3-}$ does not hydrolyse (as does $FeCl_3$) in aqueous media as the Fe^{3+} are strongly bound in the cyanide complex. Hence no changes of membrane resistance occurred when $K_3[Fe(CN)_6]$ was added to the electrolyte. This is in contrast to $FeCl_3$ which can produce great resistance changes in lipid bilayers as reported by McDonald and Thompson[16].

RESULTS

The polarity of the photosignals was defined in the following way: When compartment 1 of the cell containing the ferricyanide solution (Fig. 1) was positive with respect to the ferricyanide-free solution in compartment 2 a positive voltage developed. The signal on top of all graphs (Figs. 2–4 and 6–11) shows the length of the light pulse applied to the bilayer in the photoexperiments.

In order to determine the influence of the possible sources of artifacts that could have obscured the measurements made with the pigmented membranes, the photoresponse of the Ag–AgCl electrodes as well as that of the redox system were determined separately. Pure lecithin membranes (0.5% L-α-dioleoyllecithin in *n*-decane) were used in these experiments to exclude any influence of retinal. Otherwise, the experimental conditions were identical to those under which the pigmented membranes were investigated.

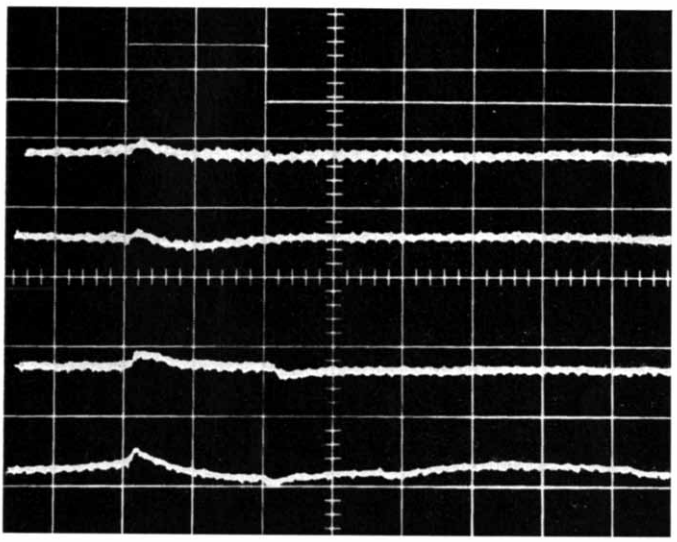


Fig. 2. Photovoltages across pure lecithin bilayer (no redox agent present) measured at different wavelengths. From top to bottom: $\lambda = 405$ nm, 366 nm, 313 nm, 265 nm. 200 μV/cm, 2 s/cm.

The photoresponse of the electrodes was measured by applying light pulses to pure lecithin bilayers at various wavelengths. No redox agent was added to the electrolyte. Fig. 2 shows a typical response of the open-loop photovoltage. The measurements were made successively and the signals were stored on the screen of a storage scope. The small photosignals were almost buried in the membrane noise; their amplitude was smaller than 50 μV at all wavelengths (Hg lines) and the time response was distinctly different from the response measured with pigmented bilayers (see below). These spurious signals were due to stray light reaching the electrodes[15]. They did not influence the measurements of the much larger photosignals generated by the pigmented membranes.

The photoresponse of $K_3[Fe(CN)_6]$ was checked after the redox system was added to the electrolyte on the left of a pure lecithin bilayer (Fig. 1). To enhance the effect the redox concentration was made 5 times higher (10^{-2} M) than in the retinal experiments. Fig. 3 shows the open-loop photovoltage which developed across the membrane when three light pulses ($\lambda = 366$ nm) of 1 s duration were applied in succession. The photovoltage did not stop decreasing right at the end of a light pulse but dropped for about another 2 s. It then increased again and finally reached a quasi stationary value which was more negative than the membrane potential before the light pulse had been applied. This light-induced offset voltage decreased with increasing length of illumination (Fig. 3). It slowly increased to zero in the dark.

Fig. 4 shows the open-loop photovoltage measured at different wavelengths (Hg lines) across a retinal–lipid bilayer. Compartment 1 of the measuring cell contained $2 \cdot 10^{-3}$ M $K_3[Fe(CN)_6]$. At 546 nm (Fig. 4) no photosignal was measured. The photovoltage was negative at all wavelengths in Fig. 4 except at 366 nm, and a negative stationary value was reached only after several minutes of illumination. The sign as well as the time response of the negative photopotential were identical

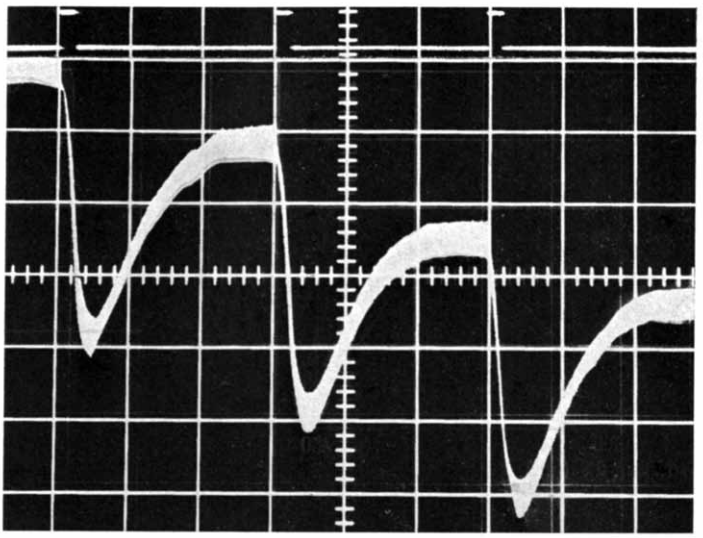


Fig. 3. Time response of photovoltage across pure lecithin bilayer (10^{-2} M K_3 $[Fe(CN)_6]$ on one side of membrane) to 3 successive light pulses of 1 s duration. $\lambda = 366$ nm, 200 μV/cm, 5 s/cm.

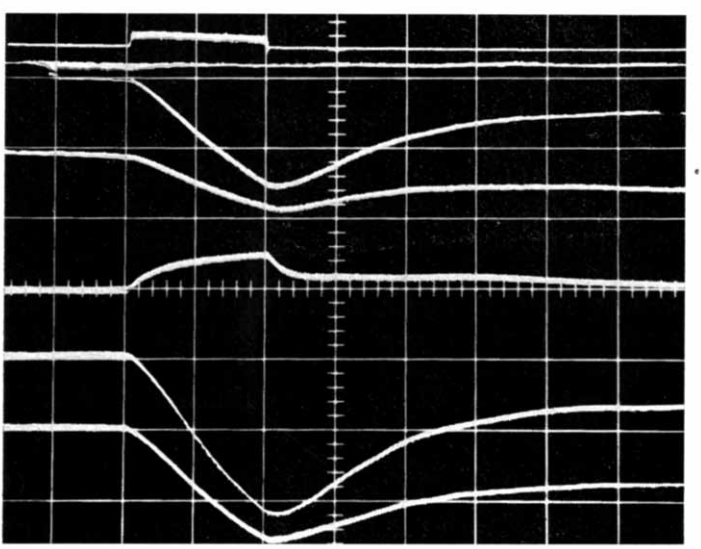


Fig. 4. Photovoltages across retinal–lipid bilayer ($2 \cdot 10^{-3}$ M $K_3[Fe(CN)_6]$ on one side of membrane) measured at different wavelengths. From top to bottom: $\lambda = 546$ nm, 436 nm, 405 nm 366 nm, 313 nm, 265 nm. 3 mV/cm, 5 s/cm.

to the voltage which developed across a pure lecithin membrane (Fig. 3). Therefore, these signals cannot be attributed to photoprocesses in the retinal membrane; they must be due to the redox system.

At 366 nm, which is close to the absorption maximum of retinal, as well as close to the absorption minimum of $Fe^{III}(CN)_6^{3-}$ (Fig. 5), the sign of the photovoltage was reversed (Fig. 4). Its time response was distinctly different from the

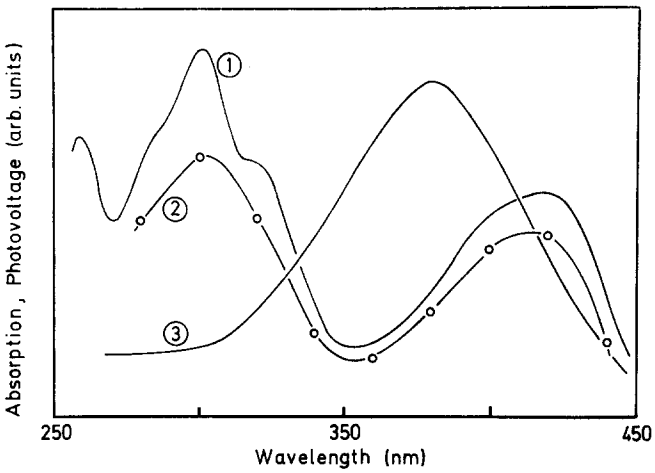


Fig. 5. Absorption spectra of $K_3[Fe(CN)_6]$ (Graph 1) and retinal (Graph 3) in solution. Photovoltage across pure lecithin bilayer (Graph 2) contacted on one side with 10^{-2} M $K_3[Fe(CN)_6]$,

response of the negative signals measured at the other wavelengths. A positive photovoltage was only generated in bilayers doped with vitamin A, and only in the wavelength range from about 340 nm to 380 nm. Outside this range the negative signals dominated, thus indicating that the signal at 366 nm was due to photoprocesses in the retinal.

Fig. 5 shows measurements of the absorption spectra of $K_3[Fe(CN)_6]$ and retinal in solution (Graphs 1 and 3, respectively). Graph 2 is a plot of the negative photovoltage measured across a pure lecithin bilayer at different wavelengths with one side of the bilayer being in contact with 10^{-3} M $K_3[Fe(CN)_6]$. The photovoltage is roughly proportional to the absorption spectrum of $Fe^{III}(CN)_6^{3-}$, a result confirming the findings that the negative photoresponse in the previous measurements (Figs 3 and 4) was an artifact due to the redox system. Only those photosignals measured close to the absorption maximum of vitamin A could be correlated to photo-processes in the pigmented membranes.

The primary photo-process in the redox system is the aquation of $Fe^{III}(CN)_6^{3-}$ (refs 17, 18)

$$Fe^{III}(CN_6)^{3-} + 2H_2O \xrightarrow{h\nu} Fe^{III}(CN)_5(H_2O)^{2-} + HCN + OH^- \quad (1)$$

It follows from the right-hand side of Eqn 1 that this photoreaction causes the pH of the ferricyanide solution to increase. As a consequence, compartment 1 of the cell (Fig. 1) which contains the ferricyanide solution becomes negative with respect to the ferricyanide-free solution in compartment 2. Therefore, Reaction 1 leads to the development of a negative photovoltage across the membrane. This was confirmed experimentally (Fig. 3) and is in accordance with the definition of the sign of the photovoltage given at the beginning of this section.

The fact that Reaction 1 had taken place was checked by measuring the pH of an aqueous solution (10^{-2} M) of $K_3[Fe(CN)_6]$ illuminated with a mercury lamp. The pH increased as expected and reached a stationary value within a few minutes. After the light was switched off the pH decreased again. According to Moggi *et al.*[17]

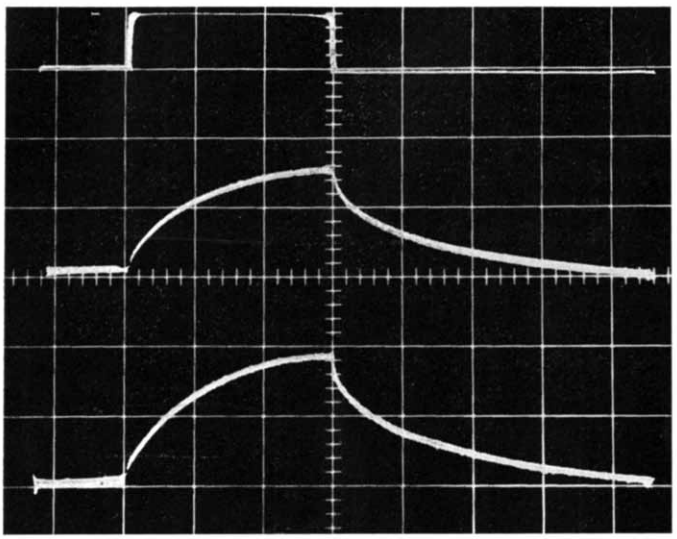


Fig. 6. Time response of photovoltage (retinal–lipid membrane) to 6-s light pulse. $\lambda = 366$ nm 1 mV/cm, 2 s/cm. Upper trace: $V_M = 0$ mV, lower trace: $V_M = +50$ mV, retinal–lipid membrane,

this can be explained by an oxidation–reduction reaction taking place as soon as $Fe^{III}(CN)_5(H_2O)^{2-}$ is produced. This second reaction lowers the pH of the solution. The measurements of the time response of the negative photovoltage (Figs 3 and 4) are qualitatively in agreement with the pH changes due to the photoreactions in the redox system.

The measurements shown in Figs 6, 7 and 8 were made with three different retinal bilayers at 366 nm. Fig. 6 shows two successive measurements of the open-loop photovoltage. The upper trace was recorded after the membrane potential V_M (a few millivolts) was, in the dark, exactly compensated by the external voltage source V_0 (Fig. 1). The experiment shows that the photopotential was due to the photogeneration of charge carriers in the retinal membrane and was not caused by a light-induced change of the membrane resistance R_M. (A light-induced change of R_M might simulate the generation of a photovoltage if the dark potential across the membrane, which generally consists of an electrode and a diffusion potential, was not zero).

The lower trace in Fig. 6 shows a measurement made to determine the influence of a d.c. membrane potential V_M on the photopotential. The amplitude of the photovoltage increased at $V_M = +50$ mV compared with $V_M = 0$ mV. At negative membrane potentials it slightly decreased. This voltage dependence indicates the photogeneration of positive charge carriers. The time response of the photovoltage was always exponential. No biphasic behaviour was found. (Fig. 6 shows only the time response of the amplitude of the photovoltage; the externally applied constant d.c. potential is not shown.)

Fig. 7 shows the open-loop photovoltage and the corresponding photocurrent. The very low current made it difficult to measure the time response. It is evident however that the current response to a rectangular light pulse is rectangular too within the time resolution of the apparatus.

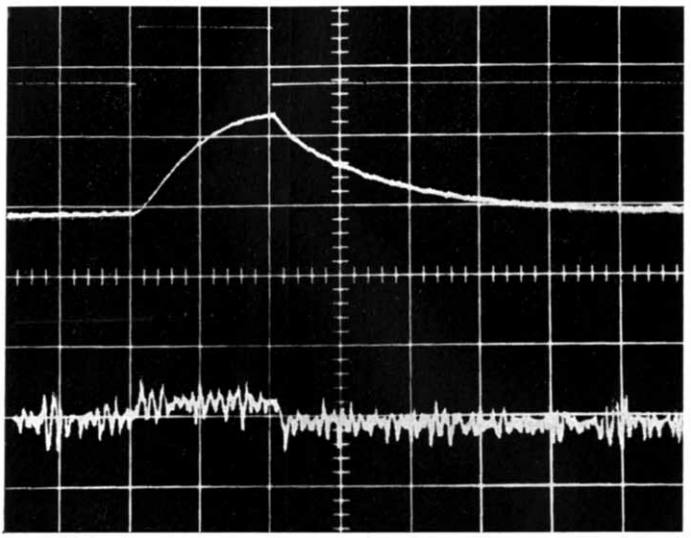


Fig. 7. Time response of photovoltage (upper trace) and photocurrent (lower trace) to 10-s light pulse. $\lambda = 366$ nm, $V_M = 0$ mV, 0.5 mV/cm, $2 \cdot 10^{-12}$ A/cm, 5 s/cm. Retinal–lipid membrane.

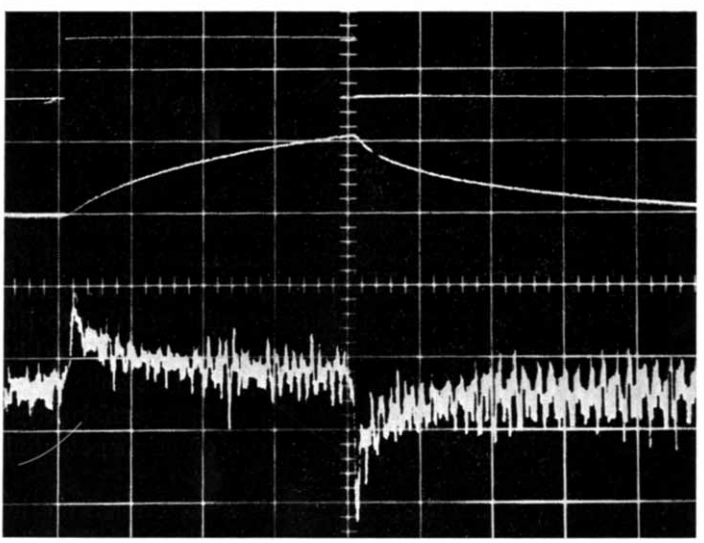


Fig. 8. Time response of photovoltage (upper trace) and photocurrent (lower trace) to 8 s light pulse. $\lambda = 366$ nm, $V_M = 0$ mV, 1 mV/cm, $5 \cdot 10^{-12}$ A/cm, 2 s/cm. Retinal–lipid membrane was illuminated for 60 s prior to experiments.

The measurements in Fig. 8 were made after the bilayer was illuminated for 60 s. The response of the open-loop photovoltage was still exponential. However, the photocurrent was no longer a simple step function, but was composed of a step function *plus* a biphasic signal which decayed exponentially to zero. When the membrane was exposed to light for an extended period of time it quite often happened that even more pronounced biphasic photosignals developed. The generation of biphasic signals in retinal bilayers was irreversible, indicating a decomposition of the retinal.

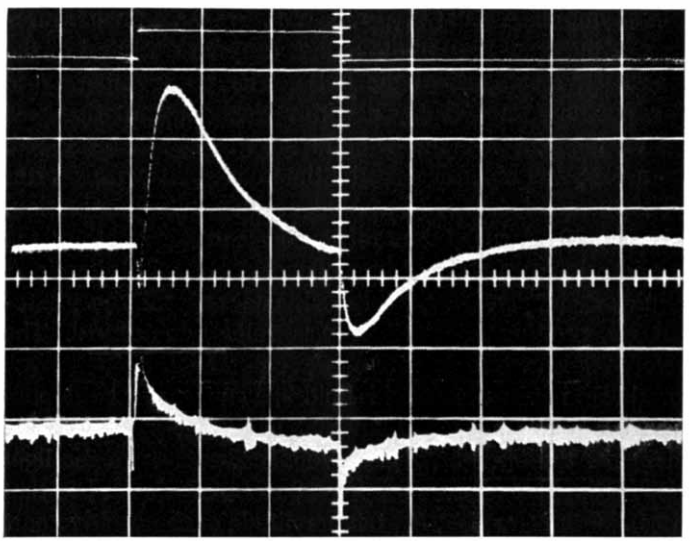


Fig. 9. Time response of photovoltage (upper trace) and photocurrent (lower trace) to 6-s light pulse. $\lambda = 366$ nm, 200 μV/cm, 10^{-11} A/cm, 2 s/cm. Vitamin A acid–lipid membrane.

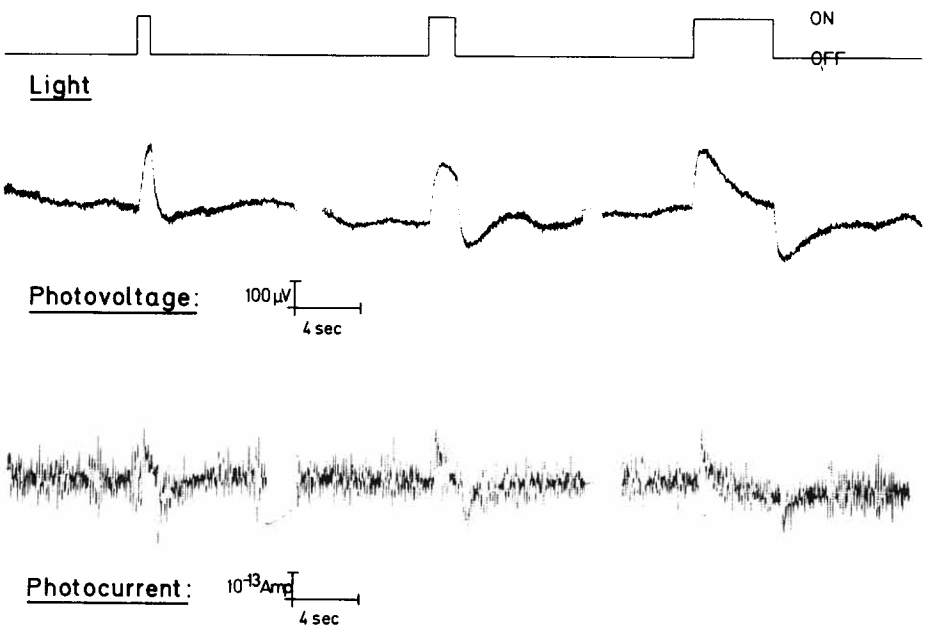


Fig. 10. Time response of photovoltage and photocurrent *versus* length of exciting light pulse ($\lambda = 366$ nm), vitamin A acid–lipid membrane.

The time response of the photosignals generated by lipid bilayers doped with vitamin A acid was very different from the response of the retinal membranes. Both open-loop photovoltage as well as photocurrent were biphasic. As with retinal membranes, photosignals could be measured only in the wavelength range close

to the absorption maximum of vitamin A acid ($\lambda_{max}=365$ nm). Fig. 9 shows measurements of the time responses of the open-loop photovoltage (upper trace) and of the photocurrent (lower trace) to a rectangular light pulse. The photovoltage rose exponentially. It reached a maximum and decayed with a time constant that was longer than the rise time. After the light was switched off a negative signal developed. The time response of the current was biphasic too; its time constants were shorter than those of the voltage. The current decayed to zero under steady-state illumination.

The measurements in Fig. 10 show the time response *versus* the length of the exciting light pulse of the photosignals generated in a bilayer doped with vitamin A acid. With increasing length of illumination the biphasic character of the photopotential became increasingly pronounced until it was fully symmetrical. The measurements in Fig. 10 were made at zero dark membrane potential.

The photovoltage produced in vitamin A acid–lipid bilayers was more dependent on externally applied d.c. potentials V_M, than that in retinal–lipid bilayers. Fig. 11 shows measurements of the open-loop photovoltage response to 3-s light pulses *versus* V_M. The shape of the photosignals for $V_M \leqslant 0$ mV was identical to that in Fig. 10 (short light pulses). With increasing V_M the amplitude of the positive part of the signal decreased and the positive photopotentials decayed rapidly during illumination (Fig. 11). The amplitude of the negative photopotentials increased.

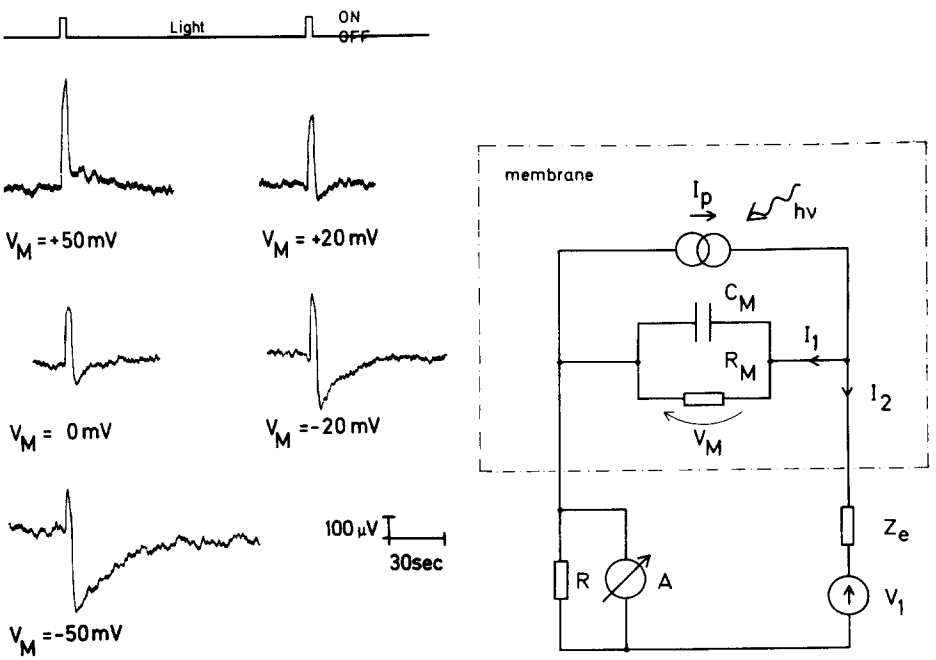


Fig. 11. Time response of photovoltage to 3-s light pulses ($\lambda=366$ nm) *versus* d.c. membrane potential V_M. Vitamin A acid–lipid membrane.

Fig. 12. Equivalent circuit simulating the time response of the photocurrent of retinal–lipid membranes. In open-loop photovoltage measurements *versus* V_1 the circuit has to be modified in such a way that the external voltage source and the high impedance of the electrometer ($R\rightarrow\infty$) are parallel (four-point probe technique).

Theory and comparison with measurements

The photogenerated signals in retinal–lipid as well as in vitamin A acid–lipid bilayers can be simulated with appropriate electrical networks. If the time responses of the output open-loop voltage and of the short-circuit current of a network to a properly applied electrical signal[19] are identical to the response measured in bilayers, then the network can be used as an analogy model. The influence of physical parameters and of the measuring apparatus on the time response can easily be discussed in terms of equivalent circuits. In the following two different analogy models will be discussed. Their time response was found to be comparable with the time response of the photoreactions proposed to explain the experiments.

(A) Retinal model

Fig. 12 shows the analogy model used to simulate the photoresponse of the retinal–lipid bilayers. This equivalent circuit was chosen among others of similar simplicity. It was the only one simulating the measured time response. The light-operated current source producing the photocurrent I_p is parallel to the membrane resistance R_M and the membrane capacitance C_M. The voltage source $V_1=(V_e-V_0)$, V_e=electrode+diffusion potential across the membrane, V_0=externally applied voltage, is in series to the complex electrode impedance $Z_e=(R_e$ parallel $C_e)$ and to the output impedance R of the instrument A (Figs 1 and 12). The instrument A was used either as an ammeter ($R \ll R_M$), or as a voltmeter ($R \gg R_M$). In the latter case V_0 was parallel R (four-point probe). V_M is the potential across the membrane. Since $R_e \ll R$, R_M and $C_e \ll C_M$ it can be shown that the impedance Z_e of the reversible Ag–AgCl electrodes does not influence the measurements of the photoresponse. (The measured time response of the electrodes was shorter than 0.5 μs). Z_e could therefore be neglected in the calculations.

The time response of the photocurrent source in Fig. 12 is assumed to be the step function $I_p=I_0\varepsilon(t)$, with $\varepsilon(t<0)=0$ and $\varepsilon(t\geqslant 0)=1$, I_0=constant. (I_p depends in reality on the photokinetics and on the external voltage V_1. Its actual time response is therefore more complicated and the current source is in reality a non-linear element.) With this assumption and provided V_1 is a constant d.c. potential one gets for the time response of the current I_2 in Fig. 12

$$I_2(t)=\frac{I_0R_M}{R+R_M}\left[1-\exp\left(-\frac{R+R_M}{RR_MC_M}t\right)\right]+\frac{V_1}{R+R_M} \tag{2}$$

The time response of the open-loop photovoltage which is measured across $R\to\infty$ (Fig. 12) can be calculated from Eqn 2

$$V_2(t)=\lim_{R\to\infty}[RI_2(t)]=I_0R_M\left[1-\exp\left(-\frac{t}{R_MC_M}\right)\right]+V_1 \tag{3}$$

Eqn 3 is in agreement with the experimental findings which show (Figs 6, 7 and 8) that the open-loop photovoltage response is exponential when a light pulse is applied to the membrane. The rise time of $V_2(t)$ is determined by the membrane resistance R_M and the membrane capacitance C_M. For symmetry reasons it is identical to the decay time. The first term of the sum in Eqn 3 does not depend on externally applied d.c. membrane potentials. This result is in agreement with the assumption that the

current source in Fig. 12 is a linear element but – due to the nonlinearity of the actual current source – it is not in agreement with the experimental findings (Fig. 6).

For the photocurrent measurements the input impedance of the instrument A (Figs 1 and 12) was $R \ll R_M$. Thus Eqn 2 becomes:

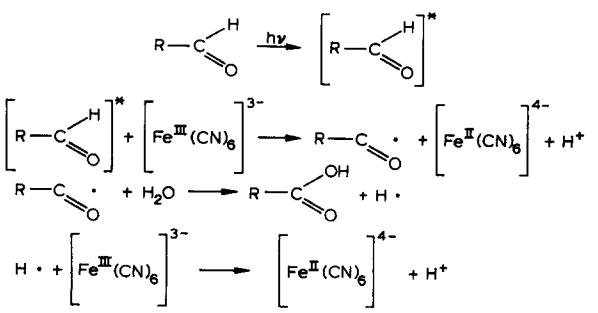
$$I_2(t) = I_0 \left[1 - \exp - \left(\frac{t}{RC_M}\right)\right] + \frac{V_1}{R_M} \tag{4}$$

The time response of the photocurrent is much faster than the response of the open-loop photovoltage because $R \ll R_M$ (Eqns 3 and 4). This is in agreement with the experiments (Fig. 7).

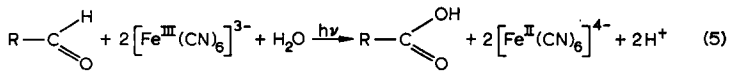
From the time response of the open-loop photovoltage (Fig. 7) the membrane capacitance C_M can be calculated by means of Eqn 3 provided R_M is known. Given the amplitude of the photocurrent and the photovoltage (Fig. 7) one gets $R_M = 7 \cdot 10^8\,\Omega$. R_M agrees well with the membrane resistance in the dark $R_M^* = 6.6 \cdot 10^8\,\Omega$ deduced from d.c. current–voltage measurements. The time constant of the photovoltage was found to be $\tau_1 = R_M C_M = 5.1$ s. This value together with R_M —both resulting from the photoexperiments— give $C_M = 7.2$ nF. C_M is in good agreement with $C_M^* = 9.0$ nF which was determined independently. (C_M^* was measured in the dark from the time response of the membrane current to a step voltage. For this purpose the d.c.-voltage source V_0 and the electrometer A were replaced by a fast square wave generator and a current–voltage converter, respectively (Fig. 1).

The input impedance of the ammeter A (Figs 1 and 12) was $R = 10^7\,\Omega$. With $C_M = 7.2$ nF one gets $\tau_2 = 105$ ms as the time constant of the photocurrent response (Eqn 4). τ_2 is the limiting parameter which determines the time resolution of the apparatus. Therefore only response times longer than 100 ms can be attributed to physical processes that occur during the photogeneration of charge carriers. Unfortunately, the photocurrents were found to be low and consequently had a poor signal/noise ratio (Fig. 7). A precise determination of τ_2 and an analysis of the fast photochemical kinetics involved was therefore not possible. It can, however, be seen in Fig. 7 that $\tau_2 \ll \tau_1$. Thus there is no contradiction between the experimental results and the current response of the analogy model in Fig. 12.

The measurements on retinal–lipid bilayers suggest that the photocurrent is generated by a photoreaction taking place at the membrane–solution interface adjacent to the redox system. Under the influence of light retinal is oxidized to vitamin A acid when an aqueous redox solution is present. The excited retinal molecules act as electron donors to $[Fe^{III}(CN)_6]^{-3}$, reducing Fe^{III} to Fe^{II}. The different steps of the photoreaction are:



The overall reaction is



(5)

When Reaction 5 takes place, the pH in Compartment 1 (Fig. 1) decreases and a positive photovoltage develops across the membrane. The retinal concentration in the bilayer decreases in the course of an experiment whereas the vitamin A acid concentration increases accordingly. The formation of vitamin A acid in retinal–lipid membranes should give rise to a biphasic photoresponse similar to the reponse of pure vitamin A acid–lipid bilayers (Figs 9–11). Indeed, at the beginning of an experiment no biphasic photosignals developed (Fig. 7). With increasing length of illumination the photocurrent became increasingly biphasic (Fig. 8) as it did in bilayers doped with vitamin A acid, a result which confirms the suggested photochemical Reaction 5. Furthermore, the photoresponse of this type of reaction can be shown to be exponential[5] when a light pulse is applied to the membrane, provided it is not too intense to modify a considerable amount of the retinal in an irreversible manner. This is in agreement with our model calculations (Eqns 3 and 4) and the experimental findings (Figs 6–8).

It was shown[5] that when a d.c. potential $V_M \neq 0$ is applied across the membrane the photovoltage dependence of Reaction 5 becomes

$$V_2(V_M) \propto \left[1+\exp\left(-\frac{qV_M}{2kT}\right)\right]^{-1} \tag{6}$$

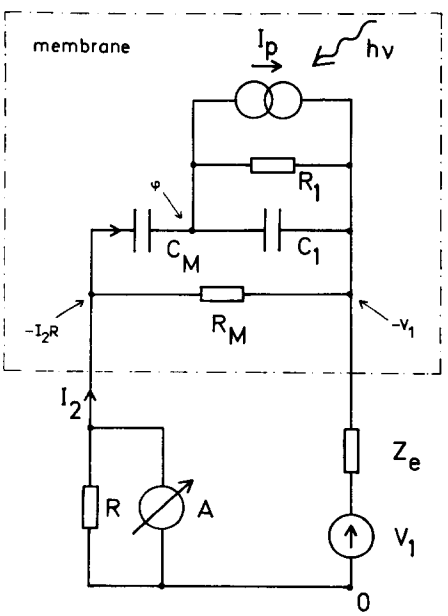


Fig. 13. Equivalent circuit simulating the time response of the photocurrent of vitamin A acid–lipid membranes. Further details as in legend to Fig. 12.

with q=elementary charge, k=Boltzmann constant and T=temperature. In our experiments $V_M = 50$ mV was the highest d.c. potential that was applied (Fig. 6). Theoretically the amplitude of the photovoltage should change by 37% when this value is inserted in Eqn 6. Experimentally a change of 31% was found (Fig. 6) which agrees well with the theory.

(B) Vitamin A acid model

Fig. 13 shows the equivalent circuit used to simulate the measured photoresponse of bilayers doped with vitamin A acid. A light-operated current source is parallel to R_1 and C_1 and in series to the membrane capacitance C_M. Otherwise the circuit in Fig. 13 is identical to the retinal model (Fig. 12). Assuming, as in model A, that the photocurrent is the step function $I_p = I_0\ \varepsilon(t)$ and that $R_1 \ll R_M$ and $C_1 > C_M$ (*i.e.* $R_M C_M \gg R_1 C_1$) —an assumption that is justified as will be shown by the comparison of the calculations with the measurements— one gets from Fig. 13

$$V_2(t) = I_0 R_1 \exp\left(-\frac{t}{R_M C_M}\right)\left[1 - \exp\left(-\frac{t}{R_1 C_1}\right)\right] + V_1 \tag{7}$$

Eqn 7 describes the time response of the open-loop photovoltage which is measured parallel to the membrane (Fig. 13). For the same reasons as in the retinal model the first term of the sum in Eqn 7 does not depend on externally applied d.c. potentials which is in contrast to the experimental findings (Fig. 13). The response of $V_2(t)$ to a step light pulse consists of an exponential increase in the voltage when the light is switched on. Its rise time is determined by R_1 and C_1. After having reached a maximum the photovoltage decays to zero with a decay-time constant $R_M C_M$. For symmetry reasons (Fig. 13) the mirror image of the positive photopotential develops when the light is switched off, *i.e.* the photopotential is biphasic.

With the assumptions $V_1 = 0$, $I_p = I_0\ \varepsilon(t)$, $R_M C_M \gg R_1 C_1$, $C_1 > C_M$ and the condition necessary for the current measurements, *i.e.* $R \ll R_M$, one gets –after some lengthy calculations— for the time response of the photocurrent

$$I_2(t) = \frac{I_0 C_M}{C_1} \exp\left(-\frac{t}{RC_1}\right)\left[1 - \exp\left(-\frac{2t}{RC_M}\right)\right] \tag{8}$$

Eqn 8 shows that the shape of the photocurrent is similar to that of the photovoltage (Eqn 7). It is also biphasic. Both rise- and decay-time are limited by the input impedance R of the ammeter. As $C_1 > C_M$ the rise-time of the current is shorter than the decay-time (Fig. 9).

Given the input impedance $R = 10^7\ \Omega$ of the ammeter, the capacitance C_1 can be calculated. From Eqn 8 and the decay-time of the photocurrent $RC_1 = 1.8$ s (Fig. 9) one gets $C_1 = 180$ nF $= 5.3\ \mu$F/cm^2. With C_1 and the rise-time constant $R_1 C_1 = 1.45$ s (Fig. 9) of the open-loop photovoltage (Eqn 7) one gets $R_1 = 8 \cdot 10^6\ \Omega$. From d.c.-current–voltage measurements the membrane resistance was found to be $R_M = 5 \cdot 10^8\ \Omega$. Together with the decay-time of the photovoltage (Fig. 9) $R_M C_M =$ 5.1 s (Eqn 7) one gets $C_M = 10$ nF. C_M is in good agreement with $C_M{}^* = 9$ nF, which was determined independently. The rise-time $RC_M = 0.1$ s of the photocurrent (Fig. 9) was limited by the time resolution of the electronics. The comparison of the experimental results with the calculated time responses of the analogy model

in Fig. 13 shows that the assumptions made in the calculations are justified. The simulated time response of the analogy model agrees well with the experimental findings.

The measurements suggest that excited vitamin A acid molecules in the membrane exchange electrons with adsorbed redox molecules upon illumination of the bilayer. A stationary dipole layer may develop under the influence of light with a large double-layer capacity C_1 which is charged during illumination. R_1 designates the "leak" resistance of the double-layer. It prevents the voltage across C_1 to rise indefinitely when a constant current is flowing (Fig. 13). The photovoltage response to the charging (light-on) and decharging (light-off) of C_1 is biphasic, in agreement with the experimental findings (Figs 9–11). The shape of the photoresponse for this type of mechanism was shown to be of the same form[4] as suggested by our model calculations (Eqns 7 and 8). The details of the mechanism leading to the formation of a Goy–Chapman double-layer adjacent to the membrane are not known. It may be that the dissociation constant K for protons of the weak vitamin A acid changes upon illumination on the side of the bilayer contacting the redox solution, thus establishing a double-layer. If it is assumed that $K^* > K$, where K^* is the dissociation constant of vitamin A acid in the first excited singlet state, then the pK-inequality $pK^* < pK_a$ holds. A transient positive photopotential develops upon illumination of the membrane until a new equilibrium is established. Assuming that such a mechanism is correct, one would expect a mirror image photosignal to develop after the light is turned off because $K^* \rightarrow K$ holds in the dark. Thus it seems possible to explain the transient nature and the sign of the measured photosignals (Figs 9–10) under the above assumption. However, whether such an explanation is correct has yet to be proved by further experiments.

DISCUSSION

The electrical analogy model used to simulate the time response of the photosignals of retinal–lipid as well as of vitamin A acid–lipid bilayers are in good agreement with the experimental findings. The only drawback of the models is that they fail to describe the measured dependence of the photosignals on externally applied d.c. membrane potentials V_M. As was shown experimentally the photogeneration of charge carriers is in reality a nonlinear process which can not be simulated by linear elements. However, the nonlinear V_M dependence of the photovoltage in retinal–lipid membranes can be understood when the calculations are based on the suggested photoreactions occurring in such membranes. Analogy models similar to those used in the present studies were proposed by Ullrich and Kuhn[4] and by Trissl and Läuger[5]. These authors investigated the photoresponse of lipid bilayers pigmented with cyanine dyes and chlorophyll molecules, respectively. Assuming a dielectric constant $\varepsilon = 10$ and a charge separation in the double-layer of 5 Å a capacity $C_d = 20\ \mu F/cm^2$ was estimated in chlorophyll-doped membranes[5] (within an order of magnitude). C_d compares well with the double-layer capacity $C_1 = 5\ \mu F/cm^2$ determined in the present experiments with vitamin A acid–lipid membranes.

The measurements on vitamin A acid–lipid bilayers show that the influence of externally applied d.c. potentials on shape and amplitude of the photovoltage is much more pronounced than in retinal–lipid membranes (Figs 6 and 11). A com-

parison of these results with the photoexperiments made with $FeCl_3$ as a redox solution, reported by Kobamoto and Tien[9,10], indicates that the photosignals attributed by these authors to retinal were due to vitamin A acid. If this assumption is correct then the reported influence of the pH on the biphasic shape of the photovoltage[9] can —as the above measurements in Fig. 11 show— be simulated by the application of appropriate d.c. potentials across the pigmental bilayer. As was shown (model A) retinal is oxidized to vitamin A acid in the course of a photoexperiment. This was also confirmed in an independent experiment with an aqueous solution of $FeCl_3$ and retinal exposed to ultraviolet light for several minutes. Thin-layer chromatography showed that a considerable amount of vitamin A acid was produced during the illumination.

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