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PHOTOELECTRIC EFFECTS AT LIPID BILAYER MEMBRANES: THEORETICAL MODELS AND EXPERIMENTAL OBSERVATIONS

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SUMMARY

In the first part of this paper a theory of photoelectric effects at thin lipid membranes is developed, which is based on two different models. In Model 1 it is assumed that a continuous production of mobile charge carriers takes place at one membrane–solution interface under the influence of light. In Model 2 the light leads to the formation of an electrical double layer in the interface. The time course of the photocurrent and of the photovoltage are calculated for both models, and criteria are developed for the experimental distinction between the two mechanisms.

In the second part two experimental systems are described. It is shown that the photoelectric effects at a thin chlorophyll membrane in the presence of *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD) are consistent with the first model. In this system a steady current flows through the membrane upon irradiation from the TMPD side to the TMPD-free side under short-circuit conditions, indicating a continuous photoproduction of charge carriers. The rise time of the photocurrent is less than 100 μs. An example for the second model is given by a chlorophyll membrane to which a protein such as cytochrome *c* or lysozyme is added on one side. In this case a transient photocurrent is observed which is characteristic for the generation of an electrical double layer. As predicted by the theory, the photocurrent was found to be independent of the applied voltage.

INTRODUCTION

In the last years a number of papers on photoelectric effects at lipid bilayer membranes have been published[1–12]. These studies have been undertaken mainly with the aim to get some insight into elementary reactions which may play a role in photosynthesis of green plants[1–3,6–8,10] or in the visual process in retinal rods[12]. As a possible explanation of the light-induced synthesis of ATP in the chloroplasts, it has been proposed that the primary action of light consists in the generation of an electric field across the photosynthetic membrane[13]. Indeed, experimental evidence for a light-induced change of the membrane potential in chloroplasts has been presented recently[14–16].

If pigments such as chlorophyll *a*[1–3,6–8,10], retinal[12], or cyanine dyes[5] are added to an artificial lipid membrane, photocurrents and photovoltages are observed if the

Abbreviation: TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine.

membrane is irradiated with light. In some systems, the observed photoeffects strongly depended on the addition of redox components to the aqueous solutions. The photocurrents and photovoltages usually showed a characteristic time dependence after a sudden change in the light intensity.

Up to now most of these studies were based on a purely phenomenological approach. In view of the diversity of observed effects, however, a theoretical analysis of photoelectric effects at lipid membranes is desirable. At present, a comprehensive theory of photoeffects at membranes is not yet feasible, mainly because too little is known about the details of the reaction mechanisms. In this paper, we therefore do not attempt to give such a general theory, but rather base our analysis on two special models which may be regarded as limiting cases of a real system. It appears that some of the observed photoeffects may be discussed in terms of one or the other of the two models. In the first model, it is assumed that the photoreaction consists of a continuous production of mobile charge carriers at one membrane–solution interface. In the second model, the excitation of pigment molecules in the membrane leads to the generation of an electrical double layer in the interface. In the experimental part of the paper, examples for both models are described briefly.

THEORY

Model 1: Production of mobile charge carriers

We assume that the membrane contains pigment molecules P which are excited by light. The excited pigment P* may then undergo a redox reaction with a component A of the aqueous phase. In this way an ion A^+ (or A^-) is formed in the membrane–solution interface:

$$P^* + A \rightarrow P^- + A^+ \qquad (1)$$

For the moment, we further assume that P^- returns to the ground state P by a reaction with an unspecified component of the aqueous solution, so that a continuous production of A^+ may take place as long as the membrane is irradiated (the aqueous concentration of A is assumed to be large). If A^+ is a hydrophobic ion, the potential energy curve of A^+ shows a minimum in the membrane–solution interface[17] (Fig. 1). A^+ may then jump either into the aqueous phase (rate constant k), or across the central energy barrier into the opposite potential minimum (rate constant k_1). Only the second process contributes to the photocurrent. In the presence of an electric field the rate constants for the jump from left to right (k_1') and from right to left (k_1'')

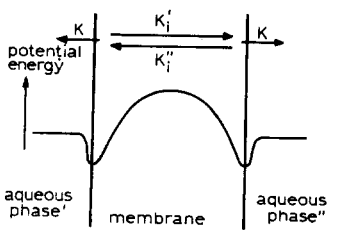


Fig. 1. Potential energy curve of the lipophilic charge carrier A^+ which is produced by the photoreaction in the membrane–solution interface. A^+ may jump from the potential minimum either into the aqueous phase (rate constant k) or into the opposite potential minimum (rate constant k_1). In the presence of an electric field the rate constants k_1' and k_1'' for the jump across the central barrier are unequal.

are different (Fig. 1). If we denote the interfacial concentrations (moles/cm^2) of A^+ by N' and N'', the photocurrent density is equal to:

$$J_{ph} = F(k_i'N' - k_i''N'') \tag{2}$$

where F is the Faraday constant. We now assume that A is present only in the left-hand solution and denote the photoproduction of A^+ in the left-hand interface by p (expressed in moles/cm^2 per s). The rate of change of N' and N'' is then given by

$$\frac{dN'}{dt} = -(k + k_i')N' + k_i''N'' + p \tag{3}$$

$$\frac{dN''}{dt} = k_i'N' - (k + k_i'')N'' \tag{4}$$

For the stationary state where dN'/dt and dN''/dt vanish, N' and N'' may be directly obtained from Eqns 3 and 4. This gives the following expression for the photocurrent:

$$J_{ph} = \frac{Fpk_i'}{k + k_i' + k_i''} \tag{5}$$

The dependence of k_i' and k_i'' on the external voltage U may be approximated by[17]

$$k_i' = k_i\, e^{u/2} \tag{6}$$

$$k_i'' = k_i\, e^{-u/2} \tag{7}$$

$$u = \frac{U}{RT/F} \equiv \frac{\psi' - \psi''}{RT/F} \tag{8}$$

where k_i is the rate constant for $U = 0$, R the gas constant, T the absolute temperature, and ψ' and ψ'' the electrical potentials of the left-hand and right-hand aqueous solutions, respectively. Eqn 5 then assumes the form

$$J_{ph} = \frac{Fpk_i}{k_i(1 + e^{-u}) + k\, e^{-u/2}} \tag{9}$$

At large positive voltages ($u \to \infty$) all charge carriers which are produced in the interface are driven across the central barrier and the current reaches the asymptotic value Fp. On the other hand, for $u \to -\infty$ the charge transport is completely inhibited ($J_{ph} \to 0$). If the aqueous phases are short-circuited ($u = 0$), the photocurrent becomes equal to

$$J_{ph}^{\circ} = \frac{Fpk_i}{2k_i + k} \tag{10}$$

If at time $t = 0$ the light is turned on, the photocurrent, measured under short-circuit conditions, rises from zero to the stationary value given by Eqn 10. $J_{ph}(t)$ may be obtained from Eqns 2–4 with $k_i' = k_i'' = k_i$. The result reads

$$J_{ph}(t) = J_{ph}^{\circ}\,(1 - e^{-t/\tau}) \tag{11}$$

$$\tau = \frac{1}{2k_i + k} \qquad (u = 0) \tag{12}$$

Thus, the three parameters of the model, k, k_1, and p, may be obtained, at least in principle, from a measurement of $[J_{ph}(u)]_{t=\infty}$ and $[J_{ph}(t)]_{u=0}$.

Under many practical conditions, the rise time of the photocurrent is limited by the parameters of the electrical circuit used to measure J_{ph}. This may be shown from the equivalent circuit of the system (Fig. 2). The photogeneration of mobile charge carriers is represented by a current source which is connected in parallel with the membrane resistance R_m and capacitance C_m. For a measurement of J_{ph}, the external resistance R_e is made negligibly small compared with R_m, so that J_{ph} is equal to U_e/R_e where U_e is the voltage across R_e. After switching on the light, J_{ph} rises exponentially with a time constant

$$\tau_e = R_e C_m \tag{13}$$

provided that the internal time constant of the current source (Eqn 12) is sufficiently small ($\tau \ll \tau_e$). The photovoltage U_{ph} is defined as the voltage across R_m at zero current under the condition $R_e \gg R_m$. If Q_m is the charge on C_m, then the rate of change of U_{ph} may be expressed by

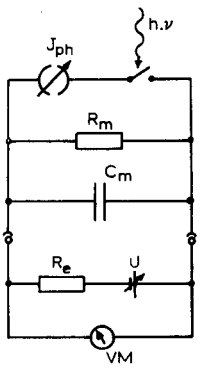
$$\frac{dU_{ph}}{dt} = \frac{1}{C_m}\frac{dQ_m}{dt} = \frac{1}{C_m}\left(J_{ph} - \frac{U_{ph}}{R_m}\right) \tag{14}$$

In the case that J_{ph} may be represented by a simple step function (τ small compared with $R_m C_m$), the solution of Eqn 14 reads

$$U_{ph}(t) = J_{ph} R_m (1 - e^{-t/\tau_m}) \tag{15}$$

$$\tau_m = R_m C_m \tag{16}$$

In the more general case where J_{ph} depends on time as well as on voltage (compare Eqns 9 and 11) the differential Eqn 14 can not be solved explicitely. Under these circumstances, however, the relation



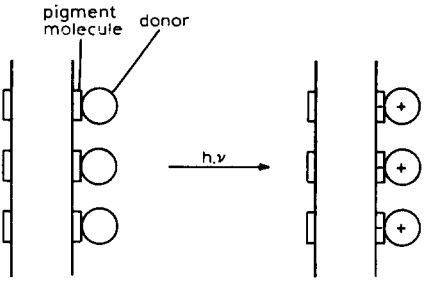


Fig. 2. Equivalent circuit for Model 1. The photogeneration of mobile charge carriers is represented by a current source which connected in parallel with the membrane resistance R_m and membrane capacitance C_m. R_e is an external resistance, U a voltage source, and VM an ideal voltmeter.

Fig. 3. Schematic representation of Model 2. Under the influence of light, a pigment molecule accepts an electron from a donor molecule which is adsorbed at one interface.

$$\left(\frac{dU_{ph}}{dt}\right)_{t=0} = \frac{I}{C_m}(J_{ph})_{t=0} \tag{17}$$

still holds which may be tested experimentally.

Model 2: Generation of an electrical double layer

The second model is defined in the following way. It is assumed that the pigment molecules are located in the membrane–solution interface, and that an excited pigment molecule may exchange an electron with a second component A which is adsorbed to one interface. In this way an array of dipoles is generated under the action of light (Fig. 3). Alternatively, the molecules A may be dissolved in the aqueous phase and may react during a collision with the interface; in this case only one half is represented by a diffuse Gouy–Chapman layer in the aqueous solution near the interface. The essential difference compared with Model 1 is the fact that no mobile charge carriers are formed, and that only a limited amount of charge may be separated.

The time course of photovoltage and photocurrent may be analyzed in terms of the equivalent circuit which is depicted in Fig. 4. When the light is turned on at time $t = 0$, the formation of dipoles in the interface sets in, and the double-layer capacity C_d is charged. The rate of the photochemical charge separation, dQ_{ph}/dt, depends on the molecular parameters of the system as well as on the light intensity, but is independent, to a first approximation, of the external voltage; this arises from the fact that the main voltage drop occurs across the hydrocarbon core of the membrane. As charge may flow from C_d to C_m across R_m and R_e, the charging rate of C_d is smaller than dQ_{ph}/dt:

$$\frac{dQ_d}{dt} = \frac{dQ_{ph}}{dt} - \frac{dQ_m}{dt} \tag{18}$$

where Q_d and Q_m denote the charges on C_d and C_m, respectively. For a measurement of the photocurrent J_{ph}, R_e is made small compared with R_m, so that J_{ph} is equal to dQ_m/dt:

$$\frac{dQ_d}{dt} = \frac{dQ_{ph}}{dt} - J_{ph} \tag{19}$$

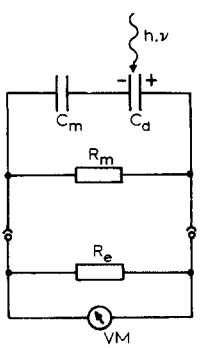


Fig. 4. Equivalent circuit for Model 2. C_d is the capacity of the electrical double layer which is formed under the action of light.

On the other hand, the voltage drop across R_e is equal to the sum of the voltages on C_d and C_m:

$$J_{ph}R_e = U_d + U_m = \frac{Q_d}{C_d} - \frac{Q_m}{C_m} \tag{20}$$

If we combine the time derivative of Eqn 20 with Eqn 19, the following relation is obtained (using $dQ_m/dt = J_{ph}$):

$$R_e C_d \frac{dJ_{ph}}{dt} = -\left(1 + \frac{C_d}{C_m}\right) J_{ph} + \frac{dQ_{ph}}{dt} \tag{21}$$

Usually, the double-layer capacity is much larger than the membrane capacity; the factor in parentheses in Eqn 21 therefore reduce to C_d/C_m. The time dependence of dQ_{ph}/dt is not known *a priori*, but it is plausible to assume that in many cases the photochemical charge production may be represented by a monomolecular reaction with a time constant τ_{ph}:

$$Q_{ph} = Q^{\circ}_{ph}\,(1 - e^{-t/\tau_{ph}}) \tag{22}$$

Q^0_{ph} is determined by the maximum number of dipoles which may be formed in the interface.

With Eqn 22, the solution of the differential Eqn 21 reads

$$J_{ph} = \frac{C_m}{C_d} \frac{Q^{\circ}_{ph}}{\tau_{ph} - \tau_e} (e^{-t/\tau_{ph}} - e^{-t/\tau_e}) \tag{23}$$

where $\tau_e = R_e C_m$, as before. This equation predicts a biphasic behaviour of J_{ph}. If $\tau_e \ll \tau_{ph}$, the photocurrent first rises with a time constant τ_e from zero to a maximum and then decreases again to zero with a second time constant τ_{ph}. Conversely, for $\tau_e \gg \tau_{ph}$, the rising phase is governed by τ_{ph} and the falling phase by τ_e.

The total charge Q_e which flows in the external measuring circuit is obtained by integration of Eqn 23:

$$Q_e = \int_0^{\infty} J_{ph}\,dt = \frac{C_m}{C_d} Q^{\circ}_{ph} \tag{24}$$

Thus, as C_m is smaller than C_d, only a fraction of the photochemically produced charge is detected in the experiment.

The photovoltage U_{ph} is measured under the condition $R_e \gg R_m$. In this case most of the current J flows through the membrane resistance R_m, so that $U_{ph} = JR_m$. U_{ph} may then directly be obtained from Eqns 18–23, if R_e is replaced by R_m. This gives

$$U_{ph} = \frac{Q^{\circ}_{ph}}{C_d} \frac{\tau_m}{\tau_{ph} - \tau_m} (e^{-t/\tau_{ph}} - e^{-t/\tau_m}) \tag{25}$$

The photovoltage shows the same biphasic behaviour as the photocurrent, but τ_e is now replaced by the much larger time constant $\tau_m = R_m C_m$. As R_m and C_m may be obtained from independent experiments, a measurement of $U_{ph}(t)$ in addition to $J_{ph}(t)$ (or *vice versa*) yields no new information.

After a prolonged period of illumination, U_{ph} (and J_{ph}) has returned to zero.

If the light is then switched off, two possibilities exist. In the case where the double layer is stable in the dark, the voltage U_{ph} remains zero. In many systems, however, the double-layer charge will disappear with time in the dark, for instance by direct recombination, or by separate reactions of both charged species with other components of the aqueous solution. In this case a current flows back from C_m to C_d. This means that a transient voltage appears across R_m which has the opposite polarity as compared with the light-on response.

EXPERIMENTAL

The experiments were carried out with (black) bilayer membranes made from a mixture of chlorophyll *a* and lecithin. There was no difference whether egg lecithin or synthetic dioleoyllecithin was used. Egg lecithin was prepared from fresh hen eggs by the method of Singleton[18]. The product gave a single spot on the thin-layer chromatogram. Dioleoyllecithin was purchased from Supelco. Chlorophyll *a* and *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD) was obtained from Fluka and was used without further purification.

The membranes were formed in the usual way on a teflon frame which was submersed in an aqueous solution and had a circular hole of 3 mm diameter. The effective area of the bilayer membrane was about 0.08 cm^2. The composition of the membrane forming solution was 0.5 % (w/v) lecithin *plus* chlorophyll in *n*-decane. If not otherwise specified, the molar ratio of lecithin: chlorophyll was 10:1.

The photoexcitation of the membrane was done by illumination with a 250-W tungsten–halogen projector lamp which gave an intensity of up to 0.3 W/cm^2 in the plane of the membrane. For spectral measurements narrow band interference filters (Balzers, Filtraflex B40) were inserted into the optical path. The energy transmitted by the individual filters was measured with a bolometer. The inverse product of the bolometer reading times the peak wavelength of the filter has been used as a correction factor for identical quantum flux densities.

The duration of the illumination was controlled with a camera shutter which had a risetime of less than 1 ms. A better time resolution was obtained with a ballistic shutter[19]. For this purpose the light beam was interrupted by a thin piece of aluminium foil; the foil was then shot away with the bullet of a gun. With this method it is possible to reach rise times of less than 5 μs.

The current–voltage characteristics were measured with an electrometer (Keithley, 610B), and for the photocurrent measurements with cytochrome *c* a storage oscilloscope Tektronix 549/1A7A was used.

RESULTS

It has been shown by light absorption and fluorescence measurements that chlorophyll *a* can be incorporated into a lipid bilayer membrane with a density of up to $3 \cdot 10^{13}$ chlorophyll molecules per cm^2, and that the porphyrin ring is accessible to reactants added to the aqueous phase [9,20,21]. Presumably, the phytol chain is inserted into the hydrocarbon part of the membrane, whereas the porphyrin ring is located in the interface.

(1) *N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD)*

Pronounced photoelectric effects are obtained if the electron donor TMPD is added to one side of a chlorophyll membrane and ascorbate to the other. The photocurrent at zero voltage, J^{o}_{ph}, and the photovoltage at zero current, U^{o}_{ph}, which are observed under these conditions are shown in Fig. 5. It is seen that after the initial rising phase, J^{o}_{ph} as well as U^{o}_{ph} approach constant values under steady illumination. Fig. 5 further shows that the experiment can be repeated many times. The measured values of J^{o}_{ph} and U^{o}_{ph}, however, depend on previous exposures of the membrane. In the first exposure only a small effect is obtained which slowly increases under continuous illumination, until, after about 10 min, a steady state is reached. Together with the action spectrum (see below) this observation indicates that the photoactive pigment is a derivative of chlorophyll *a*, which is formed during the illumination of the membrane.

The polarity of the photovoltage is such that the TMPD side becomes allways negative with respect to the TMPD-free side in the light, independent of the direction of the light. The sign of the photovoltage agrees with the assumption that the hydrophobic radical cation $TMPD^{+}$ (Wursters blue) is formed during the photoreaction of TMPD with the excited pigment:

$$TMPD + P^{*} \rightarrow TMPD^{+} + P^{-} \qquad (26)$$

$TMPD^{+}$ may then jump to the opposite solution according to Model 1, so that the TMPD side assumes a negative charge. Under zero-current conditions the negative potential of the TMPD side prevents further charge transport. On the other hand, with short circuited external solutions, a constant photocurrent is maintained by the photoreaction.

In accordance with Eqn 15, the rise of the photovoltage follows an exponential law with a time constant which is found to be equal to the electrical time constant

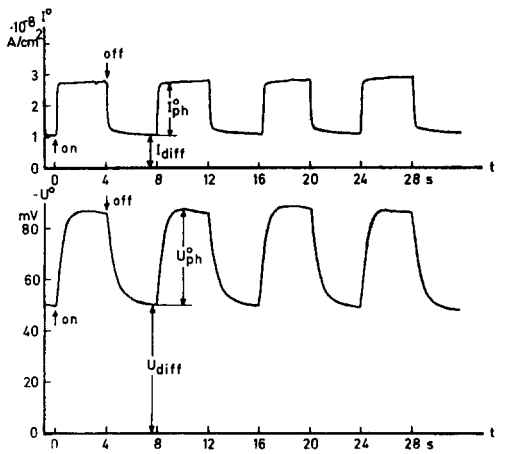


Fig. 5. Photocurrent and photovoltage of a chlorophyll membrane with 20 mM TMPD on one side and 5 mM ascorbate on the other. Both aqueous phases contained 0.1 M KCl and were buffered with 50 mM Tris to a pH of 7.8. In the dark a diffusion potential U_{diff} and a diffusion current J_{diff} are observed which arise from the asymmetry of the system. The photovoltage and the photocurrent are defined by $U^{\circ}_{ph} = U^{\circ} - U_{diff}$, and $J^{\circ}_{ph} = J^{\circ} - J_{diff}$, respectively. The rise time of the current is limited by the recorder. Light intensity = 300 mW/cm².

$\tau_m = R_m C_m$ of the membrane. The rise time of the photocurrent has been measured using the ballistic shutter. Under all conditions, the rise time was limited by the electrical time constant $\tau_e = R_e C_m$ of the measuring circuit. Therefore only an upper limit $\tau \leqslant 100\ \mu s$ can be given for the true rise time of the photocurrent.

In Fig. 6 the action spectrum of the photocurrent, corrected to constant quantum flux, is compared with the absorption spectrum of chlorophyll *a*. It is seen that the peaks of the action spectrum agree reasonably well with the absorption peaks of chlorophyll *a*. Between 500 an 600 nm, however, a difference in the relative heights of the two curves is observed. As mentioned above, this finding may be taken as evidence that the photoactive species is a derivative of chlorophyll *a*.

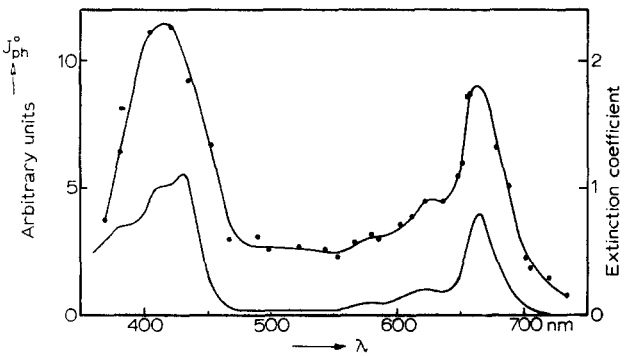


Fig. 6. Action spectrum of the photocurrent (arbitrary units, ●—●) under the same conditions as described in the legend of Fig. 5. The action spectrum is corrected to equal quantum flux. ———, absorption spectrum of chlorophyll *a* in *n*-decane.

Qualitatively the same photoeffects, but with smaller amplitude, are observed without the addition of ascorbate to the TMPD free solution. The action of ascorbate may be explained in the following way. At the pH values used in the photoexperiments (pH 7–8), TMPD is present in the unprotonized form, the pK values being $pK_1 = 2.2$, $pK_2 = 6.4$. The unprotonized molecule is highly membrane permeable (flux measurements gave an apparent permeability coefficient of $P \simeq 1.5 \cdot 10^{-4}$ cm/s; as this is in the order of the permeability of the unstirred layers near the membrane surface, the real value of P may be much higher). Hence, one has to assume that the TMPD concentration on the opposite membrane surface differs from zero, which reduces the asymmetry of the system and therefore diminishes the photoeffects. It has been found that ascorbate acts as a quencher of the photoreaction between chlorophyll and TMPD, presumably by reducing $TMPD^+$ back to TMPD. This can be demonstrated by adding ascorbate to the same side as TMPD, in which case the photocurrent vanishes. For this reason, ascorbate, if added to the opposite solution, can be used to maintain the asymmetry of the system.

In order to measure the photocurrent J_{ph} as a function of voltage U, the current–voltage characteristics of the membrane in the light and in the dark have been determined (Fig. 7). The experimental values of $J_{ph}(U)$ may be compared with the theoretical predictions of the model, which has to be modified slightly in order to account for the fact that the concentration N'' of the permeable ion ($TMPD^+$) in the

right-hand interface is zero due to the presence of the quencher. If $N'' = 0$ is introduced into Eqns 3 and 4, the result reads, instead of Eqn 9:

$$J_{ph} = \frac{Fp}{1 + (k/k_i)\, e^{-u/2}} \tag{9a}$$

The dashed line in Fig. 7 represents the theoretical curve based on Eqn 9a with $Fp = 1.9 \cdot 10^{-8}$ A/cm^2 and $k/k_i = 0.5$. The theoretical curve fits the asymptotic behaviour for $U < 0$, but a major deviation occurs at positive values of U. This indicates that additional effects play a role at large positive voltages, which are not included in the model.

That the actual mechanism of the photocurrent is more complicated than the simple model may also be seen from Fig. 8 in which J^0_{ph} is plotted as a function of the TMPD concentration c in the aqueous phase. Up to $c = 5$ mM, J^0_{ph} is proportional to c^3. A possible explanation of this behaviour is that the membrane-permeable ion is not $TMPD^+$ itself, but the complex $(TMPD)_3^+$ which may be formed in the interface by the reaction $TMPD^+ + 2\ TMPD \rightarrow (TMPD)_3^{+}$*.

The photocurrent is found to be proportional to the light intensity W up to $W = 300$ mW/cm^2. This means that saturation effects which could arise, for instance, from a slow return of the reduced pigment to the ground state ($P^- \rightarrow P$) are absent.

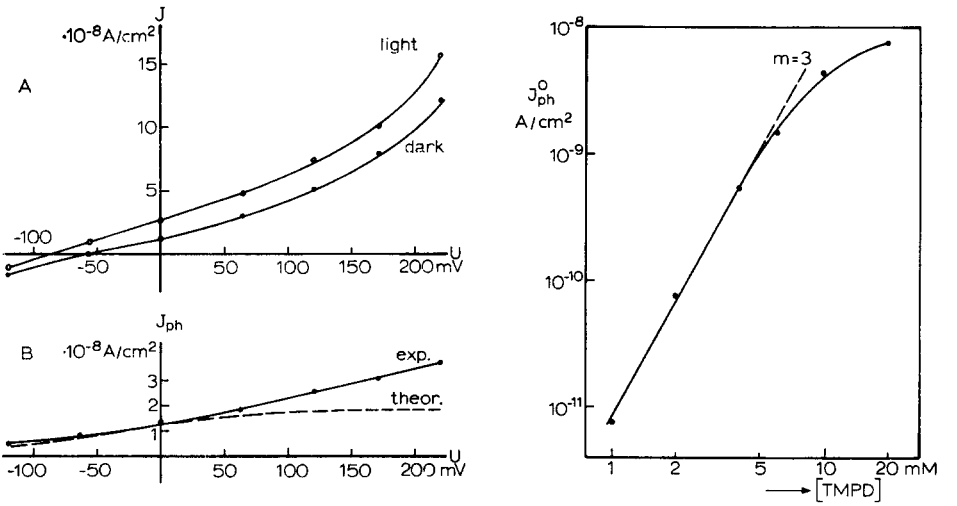


Fig. 7. A. Current–voltage characteristic of chlorophyll–lecithin membrane in the light and in the dark. 20 mM TMPD on one side, 0.5 mM ascorbate on the other and 10 mM Tris on both sides. B. Photocurrent $J_{ph} = J_{light} - J_{dark}$ as a function of voltage. Dashed line: theoretical curve according to Eqn 9a with $Fp = 1.9 \cdot 10^{-8}$ A/cm^2 and $k/k_i = 0.5$. White light (300 mW/cm^2).

Fig. 8. Photocurrent at zero voltage as a function of the TMPD concentration in the aqueous phase. 0.1 M KCl and 30 mM Tris, pH 7.8, on both sides. White light (300 mW/cm^2).

* A similar complex of the structure AD_2 forms between the electron donor hexamethylbenzene (D) and the electron acceptor tetrafluoro-*p*-benzoquinone (A) in a medium of low dielectric constant[22].

(2) *Cytochrome c*

In experiments with chlorophyll–lecithin membranes, in which oxidized cytochrome *c* has been added to one aqueous phase, photoeffects of the type predicted by Model 2 were observed. In Fig. 9 the time course of the photovoltage U_{ph} is shown for two different light intensities. The initial rate of change of the voltage is found to be roughly proportional to the light intensity. At the higher intensity, the rise time τ_{ph} of the photovoltage is about 4 s. After the light has been switched off, U_{ph} decays with a time constant of 12 s which is identical within experimental limits with the time constant $\tau_m = R_m C_m$ of the membrane.

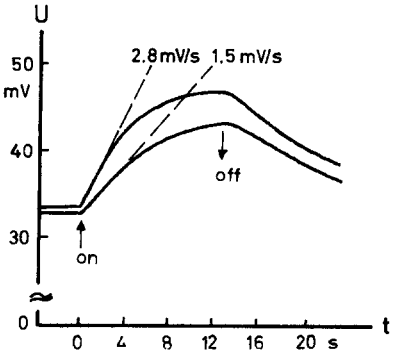


Fig. 9. Photovoltage as a function of time for a chlorophyll–lecithin membrane in $3 \cdot 10^{-4}$ M NaCl to which oxidized cytochrome *c* ($5 \cdot 10^{-5}$ M) has been added on one side. The dark voltage of about 33 mV results from the aymmetry of the system. The experiment has been performed at two light intensities which differ by a factor of 2. The initial rate of change of U is nearly proportional to the light intensity. The cytochrome *c* solution becomes more positive during the illumination.

An oscillogram of the photocurrent, measured with steady light, is represented in Fig. 10. The current rises with a time constant approximately equal to $\tau_e = R_e C_m = 2\ M\Omega \cdot 30\ nF = 0.06$ s. After reaching a maximum, the current decays with a second time constant of about 3 s, which is of the same order as the rise time τ_{ph} of the photovoltage. If the illumination is repeated after a short (1 min) period of darkness the same shape of $J_{ph}(t)$ is observed with only slightly decreased amplitude.

The above experimental findings are in agreement with Model 2 which predicts (in the case $\tau_e \ll \tau_{ph} \ll \tau_m$) that the rise time of the photovoltage should coincide with the decay time of the photocurrent. In the same way, the rise time of the photocurrent should be equal to $\tau_e = R_e C_m$ ($\tau_{ph} > \tau_m > \tau_e$). Furthermore, the quantity Q^0_{ph}/C_d may be obtained in two different ways, either by integration of $J_{ph}(t)$ (Eqn 24), or from $U_{ph}(t)$ using Eqn 25. It is found that both values of Q^0_{ph}/C_d agree within a factor of 2.

The numerical value of $\int J_{ph} dt$, evaluated from Fig. 10, is equal to approx. $1.1 \cdot 10^{10}$ elementary charges /cm². According to Eqn 24, this must be equal to $Q^0_{ph} C_m / C_d$. A rough estimation (to the order of magnitude) of C_d may be obtained using $\varepsilon = 10$ for the dielectic constant and $\delta = 5$ Å for the layer distance of double-layer capacity; this gives $C_d = 20\ \mu F/cm^2$. With $C_m = 0.4\ \mu F/cm^2$, Q^0_{ph} becomes $6 \cdot 10^{11}$ elementary charges per cm², corresponding to a mean distance of 130 Å between the dipoles.

A characteristic difference between Models 1 and 2 is the voltage dependence

of the photocurrent. Whereas in the TMPD system J_{ph} is a characteristic function of the external voltage U (Fig. 7), no voltage dependence of J_{ph} has been found with cytochrome c in the aqueous solution up to $U = \pm 80$ mV.

In order to compare the photoeffects at different membranes or at the same membrane under different conditions, the decreasing part of $J_{ph}(t)$ has been extrapolated to $t = 0$. The extrapolated value J^*_{ph} may be used as a measure of the photoresponse of the membrane. From Eqn 23 it is seen that the following relation holds:

$$J^*_{ph} = \frac{C_m}{C_d} \frac{Q^\circ_{ph}}{\tau_{ph}} \tag{26}$$

For the determination of the action spectrum, J^*_{ph} has been measured at different wavelengths. The values of J^*_{ph} which are corrected to equal quantum flux are plotted in Fig. 11 and are compared with the absorption spectrum of chlorophyll *a* in *n*-decane. It is seen that the action spectrum closely resembles the absorption spectrum of chlorophyll *a*. This means that charge separation occurs *via* excitation of chlorophyll *a*.

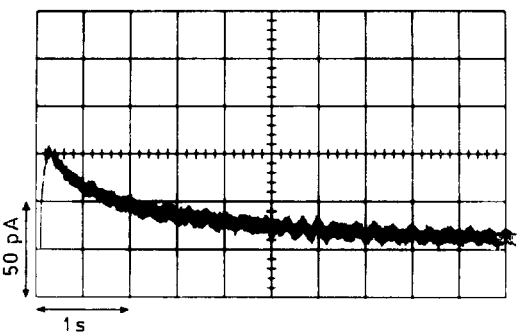


Fig. 10. Oscillogram of the photocurrent in the cytochrome *c*–chlorophyll system under the same conditions as in Fig. 9. At time $t = 0$ steady light has been switched on. The current has been measured as a voltage drop across the input resistance of the oscilloscope ($R_e = 2 \cdot 10^6\,\Omega$); the membrane resistance is higher by three orders of magnitude.

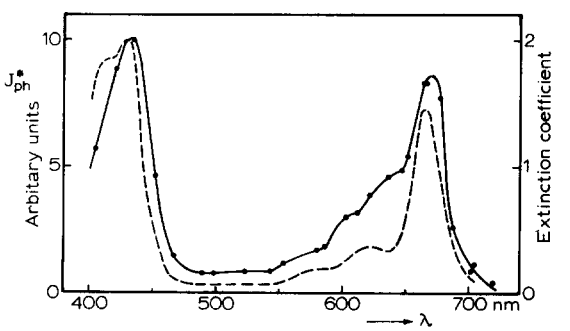


Fig. 11. Action spectrum of the photoreaction between oxidized cytochrome *c* and chlorophyll *a*. Ordinate: extrapolated value J^*_{ph} of the photocurrent (Eqn 26) corrected to equal quantum flux, in arbitrary units. The dashed line represents the absorption spectrum of chlorophyll *a* in *n*-decane. Molar ratio (chlorophyll:lecithin) = 1:3 in the film-forming solution.

Within the experimental limits, J^*_{ph} is found to be proportional to the light intensity W up to $W = 250$ mW/cm² (white light). The dependence of J^*_{ph} on the cytochrome concentration c has been studied at two ionic strengths (Fig. 12). At the lower ionic strength (10^{-4} M), J^*_{ph} is found to be independent of c down to $c = 5 \cdot 10^{-7}$ M (lower concentrations could not be used, because then the time for the development of a stationary photoresponse after the addition of cytochrome became too long). At the higher ionic strength (10^{-1} M), J^*_{ph} slowly increases with the cytochrome concentration. Interestingly, J^*_{ph} dependends strongly on the ionic strength. This is shown in more detail in Fig. 13 in which J^*_{ph} is plotted as a function of [NaCl] at a constant cytochrome concentration. It is seen that J^*_{ph} decreases sharply at [NaCl] $> 5 \cdot 10^{-4}$ M.

The mechanism of the chlorophyll–cytochrome reaction is not known in detail. As cytochrome c is likely to be adsorbed on the aqueous side of the interface, the sign of the photovoltage (the solution of oxidized cytochrome c becomes more positive during illumination) is compatible with the assumption that an electron is transferred from cytochrome c to the excited chlorophyll molecule. This excludes the possibility that the heme group of cytochrome c is involved in the photoreaction (the reverse

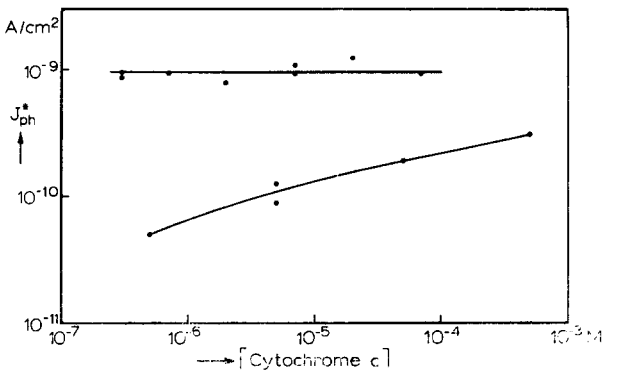


Fig. 12. J^*_{ph} as a function of cytochrome c concentration at two ionic strengths. Upper curve: 10^{-4} M; lower curve: 10^{-1} M.

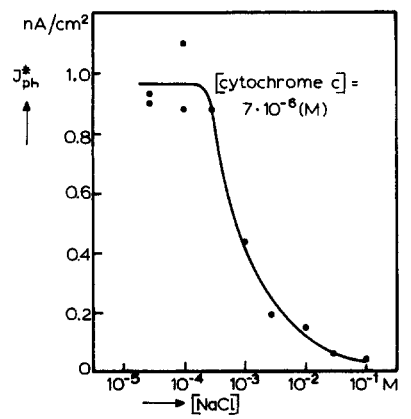


Fig. 13. J^*_{ph} as a function of **ionic strength at a constant** cytochrome concentration of $7 \cdot 10^{-6}$ M.

sign of the photovoltage had to be expected, if cytochrome is reduced). To study this question further, a few experiments have been done in which the reducing agent dithiothreitol (10^{-2} M) has been added on both sides of the membrane. In this solution cytochrome *c* is present in the reduced form. The photovoltage and the photocurrent had the same sign and nearly the same amplitude as with oxidized cytochrome *c*. The only difference was that after switching off the light, the photocurrent reversed the sign before decaying to zero. As shown earlier, this behaviour has to be expected if the light-generated double layer is unstable in the dark.

From the above experiments with oxidized and reduced cytochrome *c* it is likely that the protein moiety of cytochrome *c* is involved in the redox reaction. This conclusion is further illustrated by the finding that similar photoeffects may be produced with other proteins, *e.g.* lysozyme or serum albumin.

DISCUSSION

In this study, photoelectric phenomena at chlorophyll membranes have been investigated under two different experimental situations. If the electron donor TMPD was present in one aqueous phase, photocurrents and photovoltages are observed which approach finite stationary values under steady illumination. In the second series of experiments in which cytochrome *c* has been added to one side of the membranes, transient photocurrents and photovoltages occurred. For the TMPD system a model has been proposed in which a continuous production of membrane-permeable charge carriers takes place at one interface. The cytochrome *c* system, on the other hand, can be described by the assumption that an array of fixed dipoles is formed at the interface under the action of light. Both models may be distinguished experimentally by the time course of photocurrent and photovoltage. Furthermore, in Model 1 (production of mobile charge carriers) the photocurrent is a function of the external voltage, whereas in Model 2 the photocurrent is independent of voltage. These predictions have been verified experimentally.

Model 2 is identical with a model which recently has been proposed by Ullrich and Kuhn[23] as an explanation of their photoelectric experiments with black lipid membranes[5]. In these experiments the aqueous solution on one side of the membrane contains a cyanine dye which is adsorbed to the membrane surface. Under steady illumination, a transient photovoltage is observed which decays with a time constant equal to the electrical time constant R_mC_m of the membrane. These findings have been explained by Ullrich and Kuhn by the assumption that the excited pigment acts as an electron acceptor, so that an electrical double layer at the interface is formed in the light.

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