BBA 75667

LIGHT-INDUCED ELECTRICAL EFFECTS IN A RETINAL BILAYER LIPID MEMBRANE

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(Received December 29th, 1970)

SUMMARY

1. Ionic selectivity of all-*trans*-retinal bilayer lipid membranes was established: proton transport can satisfactorily account for the development of the observed dark membrane potentials and no other ionic species or mechanisms are required.

2. The time course of photopotentials showed a biphasic mode which, under appropriate experimental conditions, was quite similar to that of early receptor potentials.

3. The initial fast photoresponse (R1) could be best explained as a redox potential due to the photoexcited electrons and holes which interact with water molecules and/or other electron acceptors or donors. This potential was not influenced by the concentration gradient of K^+, Na^+, and Cl^- to any extent, indicating the non-ionic origin of this photoresponse. The second slow photoresponse (R2) was identified as the diffusion potential of H^+.

4. A detailed mechanism is proposed to account for the observed light-induced biphasic responses.

INTRODUCTION

Since BROWN AND MURAKAMI[1] discovered the early receptor potential in the monkey retina, the early receptor potential has aroused the interest of visual physiologists, for this is believed to be the primary event which can be elicited from the eye[2]. An elucidation of its generation mechanism is therefore considered crucial in the understanding of the initial processes of visual excitation. The early receptor potential has the following characteristics: (i) it is unaffected by anoxia[3], (ii) it is less sensitive to the ionic environment[4,5], (iii) it is found in the degenerating rodent eye, from which no electroretinogram can be recorded[6], and (iv) the early receptor potential at room temperature consists of two components: the initial corneo-positive phase and the second corneo-negative phase, which have been termed, respectively, as R1 and R2 by CONE[7].

It has been found that wave-forms similar to that of early receptor potential can be observed in non-ocular pigmented epithelium and the leaves of green plants[8-11]. In spite of the minor differences in response time and sensitivity toward termpera-

ture variations, all photoresponses observed in these pigmented tissues exhibit essentially the same features of the early receptor potential and, hence, indicate the common mechanisms underlying its generation.

At the present time the early receptor potential has not been observed in aqueous suspensions of rhodopsin or any other pigments or with the aqueous suspension of rods and cones. This fact strongly suggests that the generation of early receptor potential requires the presence of an organized pigment arrangement in membrane structure as has been indicated in the work of HAGINS AND MCGAUGH[12,13], WALD AND BROWN[11], and by GOLDSTEIN AND BERSON[15]. However, in order to investigate generations of the early receptor potential at the molecular level it is necessary to examine changes in the membrane properties upon illumination. At present even with the sophisticated intracellular recording techniques advanced by TOYODA, *et al.*[16], it has not been possible to place recording electrodes on either side of the visual receptor membrane, and to explore the basic nature of early receptor potential, although extensive studies have been made with rod outer segments[17,18]. Excellent reviews on the photochemistry and excitation of photoreceptors have been published by ABRAHAMSON AND OSTROY[19], ARDEN[20], and by WEALE[21].

With this background, we have decided to make use of artificial bimolecular lipid membranes containing carotenoid pigments as an experimental model for the visual receptor membrane and to investigate light-induced changes in the electrical properties of these membranes. A preliminary account of our work has been published elsewhere[22]. Attempts to incorporate visual and carotenoid pigments into the bilayer system have been reported by other investigators[23–27]. Recent reviews covering the general field of bilayer (black) lipid membrane research are available[28–30,32].

MATERIALS AND METHODS

Lipid and bimolecular lipid membranes-forming solutions

The oxidized cholesterol solution used in the present work was prepared from freshly recrystallized cholesterol obtained from Eastman (Rochester, N.Y.) and *n*-octane (ref. 31). A lecithin solution was prepared by dissolving 1 g of L-α-lecithin (General Biochemicals, Chagrin Falls, Ohio) into 100 ml of *n*-dodecane (pure grade, 99 % minimum), 110 ml of oxidized cholesterol solution, and 90 ml of 1 % cholesterol in *n*-octane solution. The stock solution was kept in the freezing compartment of a refrigerator. The most satisfactory membrane forming solution used in the present work was prepared by dissolving 0.025 g of all-*trans*-retinal to 0.25 ml of a 1:1 mixture of the oxidized cholesterol solution and the lecithin solution. The membrane forming solution was freshly prepared each day since the concentration of all-*trans-retinal* was close to the saturation concentration, and the evaporation of hydrocarbon solvent often resulted in the precipitation of the retinal. The membrane stability was reduced if the solution had been kept over night.

Light sources and optical arrangement

The photoexcitation of the membrane system was achieved by illumination with a tungsten-halogen lamp (DWY, 650 W Sylvania Electric, Salem, Mass.). An Alphax shutter (Wollensak, Rochester, N.Y.) was used for controlling the duration of the illumination. The light beam emerging from the aperture of the lamp housing

passed through a heat absorbing filter (water bath of 10 cm light path), a cupric sulfate solution filter (2 cm), two colored glass filters, and a lens before reaching the chamber of membrane formation. For the control of light intensity, gray filters (Carl Zeiss, Inc., New York) were used. Except for the intensity dependence experiments, photoresponses were induced without using any gray filters. The unattenuated tungsten lamp had a luminance of $3 \cdot 10^5$ ft-lux .In some preliminary experiments, excitation of the membranes was provided by a high-intensity flash lamp (Type 1539-A, General Radio Co., Mass.) with the intensity and duration of flash ranging from $0.6 \cdot 10^6$ to $11 \cdot 10^6$ lux at 1 m and 0.8–3 μsec, respectively. The spectral sensitivity curves (action spectra) were determined in an apparatus described elsewhere[32].

pH measurements and temperature control

pH of the solutions was measured with a Beckman pH meter and the adjustment of pH was done with HCl or KOH in an acetate buffer system when the control of buffer capacity was required. The temperature of the aqueous phases was measured with a thermometer with 0.1° divisions and a range from —1° to 50°, and checked repeatedly throughout the experiments. The temperature of the chambers was controlled by running water thermostatically regulated by the use of a Haake circulator (Type Re, Berlin, West Germany), through the bottom chamber of the cell assembly. The temperature could be maintained within $\pm$ 0.05° of the set value.

Cell assembly, membrane formation, and electrical measurements

The set-up and methods used in the present study are described in detail in a recent review[32].

Artifacts

The illumination of lecithin-oxidized cholesterol solution did not show any significant photoresponses. The illumination of one calomel electrodes while keeping the other out of a light beam also did not show any photoresponses in either the absence or presence of non-pigmented membrane. In the latter case the addition of powerful electron acceptors such as Fe^{3+} to one side of the nonpigmented membrane also yielded negative results. Indeed, the illumination of one electrode even in the presence of all-*trans*-retinal membrane did not show the photoresponses either. Thus, it was concluded that the photoresponses recorded with the set-up are true photoresponses of all-*trans*-retinal membranes, free from the artifacts of electrodes and the lipid component itself. The possibility that heating of water may cause the charge density gradient across all-*trans*-retinal bimolecular lipid membranes was excluded by the observation that there was not significant change in the temperature of the aqueous phases during illumination. After a 60-sec illumination at room temperature the temperature changes were less than 0.05°.

RESULTS

Dark membrane potentials

In order to understand the mechanism of the development of the photoinduced potentials of all-*trans*-retinal bimolecular lipid membranes, it is necessary to elucidate the mechanism of generation of the dark or resting membrane potentials. For this

purpose the nature of the ionic charge carriers was examined by evaluating the transference numbers of the ions used in the present work.

Shown in Fig. 1 are changes in the dark membrane potentials due to an increase in the salt concentration in the inner chamber, which was originally set equal to that of the outer chamber (10^{-3} M solutions). HCl, KCl, and NaCl developed negative potentials. The existence of the linearity clearly indicates the applicability of the Nernst equation. With the slope of 58 mV per unit pH, the protonic diffusion potential was far greater than that of other cations and anions used in the present work. The transference numbers calculated from Fig. 2 are, respectively, 1.0, 0.53, and 0.51 for H^+, K^+, and Na^+.

In the present work Fe^{3+} were used frequently as electron acceptors. Due to the strong acidic nature of the agent, the addition of $FeCl_3$ resulted in the generation of the dark membrane potential. The magnitude of this dark membrane potential was identical to those developed by the addition of HCl. This point can also be seen in the

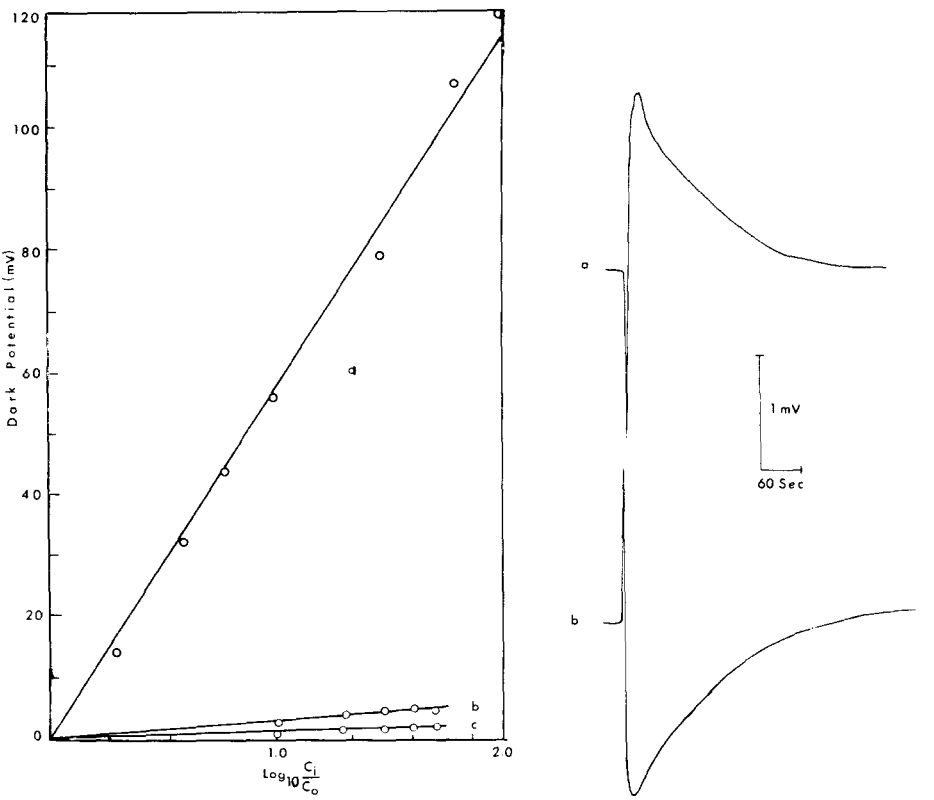


Fig. 1. Cation induced potentials as a function of concentration. The outside concentration was 10^{-3} M for all salts and the inside concentration was increased with the addition of 1 M solutions: a, HCl; b, KCl; c, NaCl; c_i, concentration in the inner chamber; c_0, concentration in the outer chamber.

Fig. 2. The effect of the location of $FeCl_3$ with respect to the incident light on the mode of photoresponse. With $FeCl_3$ (10^{-3} M) in the inner chamber the photoresponse (for 1-sec illumination) shown in a was observed while with $FeCl_3$ (10^{-3} M) in the outer chamber the photoresponse shown in b was recorded. The data indicate that the photoresponses were independent of the direction of the illuminations and solely dependent on the location of $FeCl_3$.

effect of buffer capacity on the development of the dark membrane potential due to the addition of $FeCl_3$ in symmetric acetate buffer solutions. As shown in Table I, a decrease in the buffer capacity of the aqueous phases increased the magnitude of the dark membrane potential, which could be developed by the addition of a constant amount of $FeCl_3$ in one of the chambers. The adsorption of Fe^{3+} onto the membrane may also cause the development of the membrane potential. This possibility, however,

TABLE I

THE DEVELOPMENT OF THE DARK MEMBRANE POTENTIAL AS A FUNCTION OF THE BUFFER CONCENTRATION IN THE AQUEOUS PHASES

Acetate buffer (pH 5.4); $FeCl_3$ (0.1 ml of 0.1 M) in the inside chamber. The active electrode is in the inside chamber.

Buffer concn. (M)	*Dark membrane potential (mV)*	*pH gradient*
0.1	-1.8 ± 0.1	<0.1
0.01	-31 ± 3	0.6
0.001	-110 ± 10	1.9

may be eliminated on the basis of the observation that there was no extra effect following the addition of $FeCl_3$ into the aqueous phase other than the previously mentioned pH effect observed due to the addition of HCl. The measurements of the membrane resistance also provided supporting evidence for the above point. These observations clearly establish the fact that asymmetric addition of $FeCl_3$ induces the dark membrane potentials by generating proton concentration gradient across the membrane.

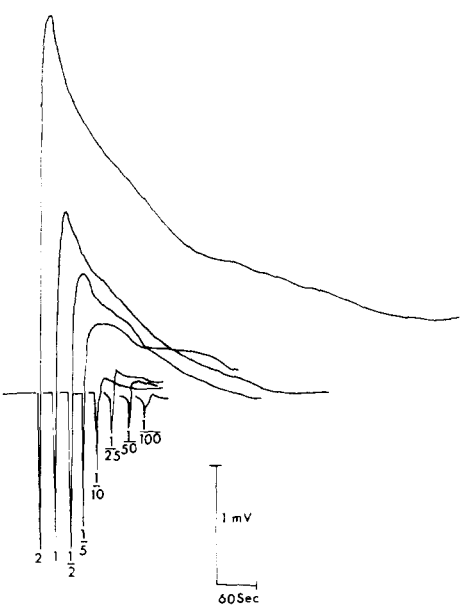
The general description of the photoresponses

The sign of the photoresponses depended upon the location of $FeCl_3$ as shown in Fig. 2a. The direction of the incident light did not influence the sign of the responses. This can be seen in Fig. 2b, which shows the photoresponse recorded with the same electrode arrangement as in Fig. 2a, but with the position of $FeCl_3$ shifted to the outer chamber in order to simulate the shift of the direction of the incident light with respect to the preceding time course curve. Unlike the cases of $FeCl_3$ and HCl, the concentration gradient of KCl and NaCl did not show any effect on the mode of the photoresponses. The 10-fold concentration gradient of KCl had no effect on the photoresponses. This fact is not unexpected since the all-*trans*-retinal bimolecular lipid membranes is not sensitive to K^+, Na^+, and Cl^-. The agreement of the mode of the effect of the salts on the photopotentials and on the dark potentials strongly suggests that the main charge carriers, or protons, in the dark state can interact with the photoexcitation of the membrane selectively, or that protons can function as charge carriers in observed photopotentials.

Due to the close similarity between the biphasic mode of the photoresponses of all-*trans*-retinal bimolecular lipid membranes and that of the early receptor potentials of photoreceptors, the convention of assigning R1 and R2 for the fast and the slow component, respectively was adapted in the present work.

The effect of the duration of illumination was investigated with 0.1 M KCl solution with 10^{-3} M $FeCl_3$ in the inner chamber. Shown in Fig. 3, a short time illu-

mination had only the R1 component and prolonging the illumination generated the R2 phase while R1 reached the apparent maximum value. The illumination time of one second for the present experiments was arbitrarily selected: at 1-sec illumination the saturation of the R1 component did occur along with the reasonably high R2 component. Indeed, the prolonged illumination caused irreversible changes on the membrane potential to such an extent that duration of illumination longer than one second was not desirable: the recovery of the initial membrane potentials was not observed as shown in Fig. 3 and 4.



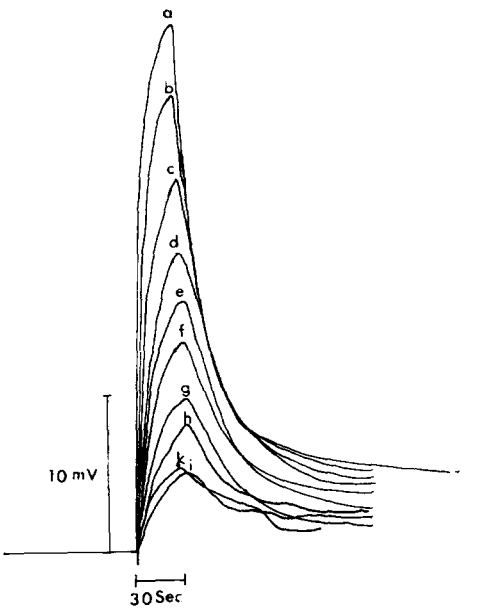


Fig. 3. The effect of the duration of illumination (in sec) on the mode of photoresponses. The photoresponses were recorded from a single membrane formed in 0.1 M KCl solution with 10^{-3} M $FeCl_3$ in the inner chamber at 25°.

Fig. 4. The effect of repetitive illuminations on the photoresponses. The letters shown on the curves refer to the times elapsed after the first illumination in min: a, 0 min; b, 10; c, 20; d, 30; e, 40; f, 50; g, 60; h, 70; i, 80; k, 155. The duration of each illumination was 30 sec.

It should be pointed out that the results given here (Figs. 2–6) do not give true pictures insofar as time variation of photopotentials is concerned owing to poor resolution of the apparatus (mechanical recorder and large RC constant of the external circuit). Some preliminary experiments using single flashes (0.8–3 μsec duration) and an oscilloscope (Type RM-503, Tektronix, Inc., Mich.) indicate that R1 component is exceeding fast and with no detectable latency (in systems containing Fe^{3+} on one side of the membrane; the rise time is in the microsecond range). The mode of generation of the photopotentials will be discussed later (see Fig. 10). These results are best explained by treating the membrane together with its biface (the two co-existing membrane/solution interfaces) as a parallel RC circuit[32] and a generator which is produced by light connected across the membrane. Thus, the charging and discharging the membrane capacitance are seen as a result of the movement of electronic and ionic charges across the membrane (see DISCUSSION).

The selection of all-*trans*-retinal as the visual pigment of the present membrane model was made partly because rhodopsin had been proved to have photoregeneration, which would complicate the mode of the photoresponses[33]. In order to examine the regeneration problem a membrane was repeatedly exposed to a long-time (30 sec) illumination and the dark membrane potential as well as the photoresponse was investigated. In Fig. 4 the effect of repetitive illuminations on the photoresponses is shown. The photoresponse decreased in its magnitude with an increasing number of illuminations, without changing the wave form appreciably. It was found that the dark potential also decreased with an increasing number of illuminations. The decrease in the dark membrane potential may indicate the generation of the photoproducts which modify the microenvironment of the membrane. If so, the decrease in the photoresponse should have a strong relationship with a decrease in the dark membrane potential, since the photoproducts cannot be formed without the consumption of the photosensitive pigment molecules, and the presence of the products may decrease the rate of photoreaction *via* product inhibition. It is interesting that the ratio of the photopotential to the dark potential decreases as the number of illuminations increases. This decrease in the efficiency of the dark potential for generation of the photopotential in an increasing number of illuminations, and the apparent lack of changes in the mode of the time course of the photoresponse with an increasing number of illuminations indicate that the regeneration problem does not exist in the present model system. The irreversible changes in the membrane potential and photoresponse due to the repetition of long-term illuminations may suggest the possible existence of the photoreaction which leads to the production of membrane active chemical species, possibly protons (see DISCUSSION).

The effect of pH on the photoresponses

The fact that resting membrane potential was solely dependent on the gradient of proton concentrations in the bathing solutions separated by the membrane immediately suggests that the mode of photoresponse should show significant variations upon changes in pH gradient. Furthermore, the possible generation of protons as one of the photoproducts may complicate the effect of pH on the photoresponses.

When pH of the outer chamber was increased by the addition of KOH, the magnitude of the slow component (R2) increased, and masking of the negative fast component (R1) could be seen unambiguously (Fig. 5).

The effect of buffer capacity on the photoresponses

Due to the essential role of the H^+ concentration gradient in determining the mode of the photoresponses, the effect of buffer capacity was also investigated. In order to study the effect of buffer capacity the proton concentration gradient has to be kept constant for various buffer capacities. This was best achieved with the use of symmetric KCl systems and symmetric acetate buffer systems. It has been found that changes in the concentration of KCl did not alter the mode of photoresponses. In acetate buffer systems, however the concentration effect was remarkable (Fig. 6). In a 10^{-3} M system R1 and R2 could be seen in exactly similar mode to those of KCl systems. Indeed, at this buffer concentration there was almost no buffering action as demonstrated in Table I. In a 10^{-2} M acetate buffer system the R2 component was completely eliminated and the R1 component was slightly enhanced. As the concen-

tration was increased to 10^{-1} M the R1 component was essentially unaffected. In order to separate the effect of buffering capacity from that of the ionic strength the concentration dependence of the dark membrane resistance was compared with the

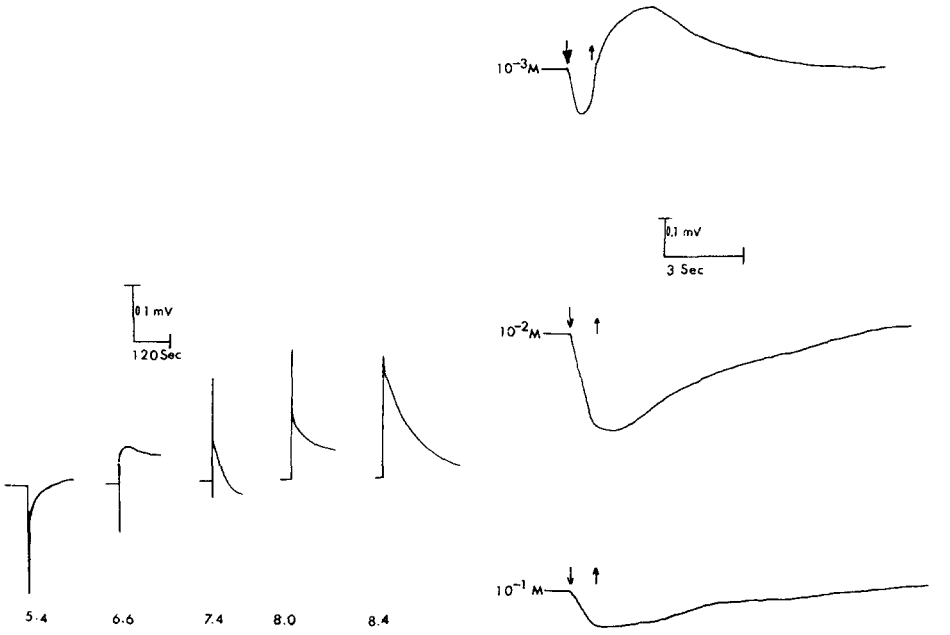


Fig. 5. The effect of pH on the photoresponses. The values of pH were those of the outer chamber adjusted with KOH and HCl in the 0.1 M KCl solution which was also contained in the inner chamber (pH 5.4).

Fig. 6. The time course of the photoresponses of all-*trans*-retinal bimolecular lipid membranes in symmetric acetate buffer solutions as a function of buffer concentration. The downward arrow indicates the light-on and the upward arrow the light-off for 1-sec illumination.

concentration dependence of the photoresponse in KCl systems and acetate buffer systems. In this comparison it was observed that the buffer capacity influenced mainly the R2 component so that in the presence of 10^{-2} M acetate buffer the complete elimination of the component was observed. The fact that this is the effect of buffer capacity can be recognized in the observation that the concentration dependence of the membrane resistance in acetate buffer systems was identical to the KCl systems, in which R2 was essentially unaffected. The effect of buffer capacity on the R2 component is strong supporting evidence for the involvement of H^+ in generation of the R2 component.

An extremely interesting observation was made on a long-time illumination. In 0.1 M KCl solution with 10^{-3} M $FeCl_3$ in the inner chamber and at zero pH gradient, a 60-sec illumination gave triphasic responses which were apparently the modified wave forms of the biphasic responses. The R1 response was taken over by the R2 component which did not pass over the resting membrane potential developed before the illumination. Instead, the R2 component bounced back and developed in the same direction as the R1 component. With a slower rate than R1, this component developed continuously up to the light-off, after which the potential decreased

instantaneously to the base line, and then slowly developed the usual R2 component passing over the base line. The final resting potential was approximately the same magnitude to the inverse R2 component but in the opposite direction. This base line shift was not reversible. In the case of high buffer capacity, the photoresponse was dramatically different. The fast developing R1 was reversed to the base line as compared with the response in the afore-mentioned unbuffered solution. After this recovery of the original potential, the reversed R2 component developed very slowly and in more suppressed manner. In addition, upon the light-off the response slowly returned to the original base line and never passed over it, unlike the response of unbuffered case.

The action spectra of carotenoid bilayers

As shown earlier[26] bilayer lipid membranes containing pigments (*e.g.* chlorophylls) give characteristic spectral sensitivity curves similar to their adsorption spectra. This was also found to be the case for the carotenoid membranes used in the present investigation (Fig. 7). The enhanced photoeffects in the presence of Fe^{3+} (or other electron acceptors) were independent of the direction of exciting light but the polarity of the induced voltages was governed by the location of Fe^{3+} being always negative with respect to the iron-free side.

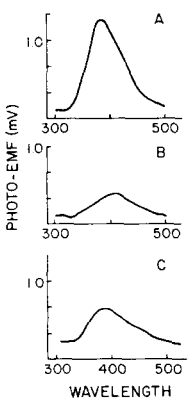


Fig. 7. The action spectra of three bilayer lipid membranes, showing the regions of absorption due to the different kinds of carotenoid pigments. A. All-*trans*-retinal alone. B. β-carotene alone. C. A 1:1 mixture of all-*trans*-retinal and β-carotene. All membranes also contained oxidized cholesterol and egg lecithin. Photo-EMF = photoelectromotive force.

DISCUSSION

General considerations

In the following paragraphs pertinent observations will be discussed to establish an overall view of the physical processes leading to the generation of the photoresponses. Especially, the reasoning for the assignment of hole diffusion potential for the R1 component and proton diffusion potential for the R2 component will be presented after establishing the mechanism of the generation of the dark membrane potentials. A detailed quantitative theory will be given in the next section, followed by the experimental verification of the theory by comparing the theoretical predictions

on the mode of the photoresponses to the experimental observations carried out in the present work.

The ionic transference numbers of KCl, NaCl, and HCl show that all-*trans*-retinal membranes are cation selective and the development of the dark membrane potentials can be explained with proton transport alone. When HCl was added in the inner chamber, the negative potential at the inner electrode developed. This may be interpreted as the proton diffusion toward the outer solution/membrane interface leaving Cl^- in the inner side. As to the question of whether the observed protonic potential is actually due to diffusion or adsorption no definitive answer can be given, since the Nernst equation is apparently valid in either case. However, if the potential were due to adsorption, the observed negative potential should be due to Cl^- but in the presence of the concentration gradient of KCl or NaCl in two aqueous phases such a large negative potential was not observed.

The elucidation of the mechanism by which this protonic potential develops is certainly interesting. In this work, however, the question was not pursued further since other evidence as presented below indicates that protonic conduction increased with water content in the hydrocarbon layer of the membrane suggesting the possibility of the contribution of water molecules to the transport of protons. The presence of the proton transport channel separated from the electronic conduction channel which is most likely to be conjugated double bonds of all-*trans*-retinal are assumed to exist in the model membranes used in the present work.

From the sign of the R1 component it is reasonable to consider that this component is likely to be either the diffusion of positive charge carriers (protons and/or holes) to the outer solution/membrane interface and/or the diffusion of electrons to the inner interface. The possibility that R1 may be mediated by protons can be eliminated by the following observations: (a) the sign of R1 was totally dependent on the position of the electron acceptors, (b) the magnitude could be greatly enhanced, as much as 10^3 times, by the addition of electron acceptors, (c) in the presence of the high concentration of electron acceptors the magnitude of R1 was independent of the proton concentration gradient across the membrane, and (d) at higher buffer capacity, where the R2 component was completely abolished, the R1 component could still be observed. Thus, our attention can be focused on electrons and holes as the charge carriers of the R1 component.

As proposed previously[26] in explaining the photopotential of chlorophyll bilayer lipid membranes, generation of R1 may be explained as follows: due to the extremely thin structure generation of electrons and holes is symmetric if there is no chemical asymmetry. On the other hand, in the presence of electron acceptors at the inner solution/membrane interface the excited electrons may tend to be withdrawn toward the inner interface, generating a higher concentration of holes in the bulk of hydrocarbon or at the outer surface.

An immediate establishment of the strong electric field across the membrane may have an effect on the space charge distribution across the membrane. The charge carriers which can be influenced by this photoinduced field are protons and holes, since electrons are likely to be trapped by Fe^{3+}, and the membrane is impermeable to Cl^-. Thus, the R2 component may be considered as the potential developed by the diffusion of protons towards the inner solution/membrane interface.

In addition to the possible interaction of protonic space charges in the genera-

tion of the R2 component, there seems to be another possibility. The accumulation of holes at the outer surface may generate protons *via* the oxidation of water molecules in the case of chlorophyll bilayers[26]. The newly generated protons contributing to pH gradient will cause a decrease in the protonic membrane potentials established at the dark conditions. Indeed, the effect of a strong buffer capacity in abolishing the R2 component cannot be explained without taking into account this photogeneration of protons. The mechanism of the photogeneration of protons is still unknown not only in the present model system but also in well studied chloroplast systems. Nevertheless, since the peak wavelength of the absorption spectrum of all-*trans*-retinal has 80 kcal/einstein and the bond energy of aldehyde–water hydrogen bonds is 2–3 kcal/mole (ref. 35), the photogeneration of protons is indeed possible energetically.

Since the membrane is impermeable to Cl^- and Fe^{3+}, the recovery of the original dark potentials can be achieved by restoration of the chemical equilibrium. Specifically, the dissociation of HCl to protons and Cl^- and the re-establishment of equilibrium between Fe^{3+} and Fe^{2+} may generate a proton gradient across the membrane and, later the diffusion of protons will re-establish the original membrane potentials. For prolonged illumination the recovery of the membrane potentials did not take place. This might be due to the disturbance of the electric double layers to such an extent that the distribution of Cl^- and Fe^{3+} is partially modified from that of the dark state due to photochemical reactions.

THEORY

In order to set up kinetic equations describing the development of the biphasic photoresponses, it is necessary to establish the physical model of the seemingly complex phenomena. The present model is based on the two basic assumptions which are shown to be valid in this work. The first assumption is that the membrane is permeable to protons, but not to the other ions studied (*e.g.* K^+ and Cl^-). The second assumption is that any external force which disturbs the existing electrochemical equilibrium has to be acted upon by a counter force to maintain microscopic electrochemical neutrality. Based on these assumptions, it is proposed that the initial electronic phenomenon following light absorption can generate the ionic charge transport, whose major charge carriers are protons[26].

In this work the resting membrane potential was expressed as the potential of the active electrode which was the inner solution/membrane interface. The photopotential was expressed as R1 and R2, which were defined as:

$$R1 = V_{min} - V_0 \quad (1)$$

$$R2 = V_{max} - V_0 \quad (2)$$

where v_{min}, v_{max}, and v_0 were the value of the membrane potentials at the first peak, the second peak and the resting (dark) state, respectively, with signs taken in the sense of the inside potential minus the outside potential. All time courses of the photoresponses could be expressed with the following two equations:

$$V = -A(1 - e^{-at}) + B(1 - e^{-bt}) \quad (3)$$

for

$0 \leqslant t \leqslant t_0$; and

$$V = -A(1 - e^{-at_0})e^{-a(t-t_0)} + B(1 - e^{-bt_0})e^{-b(t-t_0)} \quad (4)$$

for

$$t_0 \leqslant t$$

where A, B, a and b are constants and t_0 is the time (t) for the light-off taking $t = 0$ for the light-on. All constants are functions of various experimental parameters and the assignment of numerical values to them requires specification of the exact conditions under which the constants have been evaluated. Because of this it is desirable to evaluate the constants in terms of experimentally measurable parameters.

The sign of A is negative to show that the first term expresses the negative or the major component of R1 while the second term expresses the positive or the major component of R2. In spite of the fact that the observed photoresponses are the results of redox reactions and the diffusion of the products of the redox reactions or ionic charge carriers, the time courses of the photoresponses are determined by the two terms in the exponential functions. This implies either that the time constant of each component is the sum of the redox reactions and diffusion terms or that it is a pure time constant of either the redox reactions or diffusion processes. Due to the fact that these time constants (a and b) are far greater than any of the anticipated rate constants of redox reactions and diffusion processes of holes and protons, it is more fruitful to analyze the kinetic equation in terms of electric circuit theory.

The forementioned two terms of exponential functions are best expressed as the representations of the time courses of two RC circuits. Shown in Fig. 8, the hole component may be represented with Circuit 1 (Fig. 8a) and the proton component with Circuit 2 (Fig. 8b). Then, the following equations can be written down:

$$V_c^1 - V_0 = (V_1 - V_0)(1 - e^{-G_1 t/C_m}) \tag{5}$$

$$V_c^2 - V_0 = (V_2 - V_0)(1 - e^{-G_2 t/C_m}) \tag{6}$$

where V_c^1 is capacitative potential of hole component; V_c^2, capacitative potential of proton component; V_0, dark potential; V_1, saturation potential of hole component; V_2, saturation potential of proton component; G_1, conductance of hole component; G_2, conductance of proton component; C_m, membrane capacitance.

The combined system shown in Fig. 8c can be expressed as:

$$V_c^{\text{total}} - V_0 = \frac{G_1}{G_1 + G_2}(V_1 - V_0)(1 - e^{-G_1 t/C_m}) + \frac{G_2}{G_1 + G_2}(V_2 - V_0)(1 - e^{-G_2 t/C_m}) \tag{7}$$

where V_c^{total} is the total capacitative potential. Eqn. 7 expresses the time course of the membrane potential for $0 \leqslant t \leqslant t_0$. After the light-off, $t_0 \leqslant t$, the time course of the photopotential can be expressed by:

$$V_c^{\text{total}} - V_0 = \frac{G_1}{G_1 + G_2}(V_1 - V_0)(1 - e^{-G_1 t_0/C_m})e^{-G_1(t-t_0)/C_m} +$$

$$\frac{G_2}{G_1 + G_2}(V_2 - V_0)(1 - e^{-G_2 t_0/C_m})e^{-G_2(t-t_0)/C_m} \tag{8}$$

Eqns. 7 and 8 have the same properties of Eqns. 3 and 5, provided:

$$A = \frac{G_1}{G_1 + G_2}(V_1 - V_0) \tag{9}$$

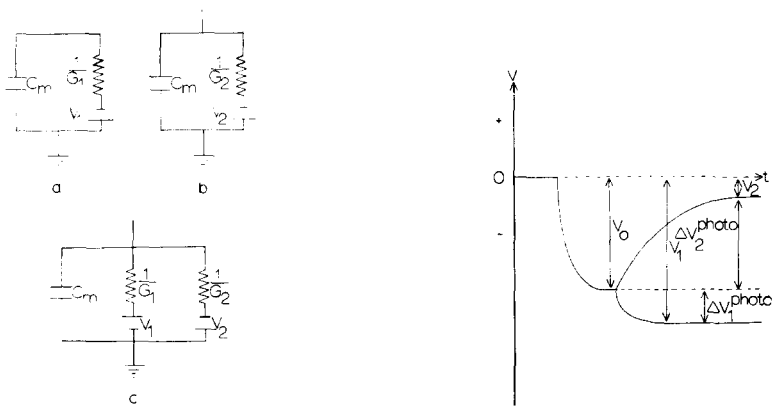


Fig. 8. The equivalent circuits for the generation of photoresponses of all-*trans*-retinal bimolecular lipid membranes. (a) Hole system designated with Subscript 1. (b) Proton system designated with Subscript 2. (c) Combined system. G, membrane conductance; V, membrane potential; C_m, membrane capacitance.

Fig. 9. The analysis of two components of photopotentials. V_0, resting membrane potential; V_1, saturation hole potential; V_2, saturation proton potential.

$$B = \frac{G_2}{G_1 + G_2}(V_2 - V_0) \tag{10}$$

$$a = G_1/C_m \tag{11}$$

$$b = G_2/C_m \tag{12}$$

Our task is now to express V_1 and V_2 in terms of other physical parameters. Since these potentials are saturation potentials, by putting $t = \infty$ in Eqns. 5 and 6 we get:

$$V_c^1 = V_1 \tag{13}$$

and

$$V_c^2 = V_2 \tag{14}$$

Referring to Fig. 9 it is possible to evaluate saturation membrane potentials in terms of the saturation concentrations of charge carriers:

$$\begin{aligned} \Delta V_1^{\text{photo}} = V_1^{\text{photo}} - V_1^{\text{dark}} &= V_1 - V_0 \\ &= \frac{RT}{F}\left\{[\ln (c_p^{i,h} + c_t^{i,h}) - \ln c_t^{o,h}] - \ln \frac{c_t^{i,h}}{c_t^{o,h}}\right\} \\ &= \frac{RT}{F} \ln \frac{c_p^{i,h}}{c_t^{i,h}} \end{aligned} \tag{15}$$

where $c_p^{i,h}$ and $c_p^{o,h}$ are the saturation concentrations of photogenerated hole in the inside surface and outside surface, respectively, and $c_t^{i,h}$ and $c_t^{o,h}$ are the saturation concentrations of thermally generated holes in the inner surface and outer surface, respectively.

Similarly we obtain:

$$\Delta V_2^{\text{photo}} = V_2^{\text{photo}} - V_2^{\text{dark}} = V_2 - V_0$$

$$= \frac{RT}{F}\left\{[\ln (c_p^{o,H} + c_d^{o,H}) - \ln c_d^{i,H}] - \ln \frac{c_d^{o,H}}{c_d^{i,H}}\right\}$$

$$= \frac{RT}{F} \ln \frac{c_p^{o,H}}{c_d^{o,H}} \tag{16}$$

where c's now stand for proton concentrations at the saturation state.

The terms expressing the concentrations must be represented by more basic parameters. In order to do this the establishment of the whole photochemical reaction starting from the absorption of light to the restoration of the original resting state of electrochemical equilibrium must be carried out. Such an attempt must introduce a great degree of simplification due to the many parameters involved in the reaction sequence, whose mathematical treatment could be unduly complicated for the proper evaluation of the experimental observations. However, there is a risk in attempting such a simplification as it may result in an unrealistic description.

The type of primary excitation species was not determined in the present work; either excitons or excited electrons could be involved. For the present discussion it is assumed that the primary excitation step is the generation of excitons[26].

The rate of exciton generation can be expressed by:

$$\frac{dc^e}{dt} = c^r \varepsilon I_0 e^{-\varepsilon c^r l} - c^e \left(\frac{1}{\tau_e} + k_1\right) \tag{17}$$

where c^e is exciton concentration, c^r is retinal concentration, ε is molar extinction coefficient I_0 is the intensity of the incident light, l is the thickness of the membrane, τ_e is the lifetime of excitons, and k_1 is the rate constant of hole generation.

At equilibrium:

$$\frac{dc^e}{dt} = 0 \tag{18}$$

Then, from Eqn. 17 we get:

$$c^e = \frac{c^r \tau_e \varepsilon I_0 e^{-\varepsilon c^r l}}{1 + k_1 \tau_e} \tag{19}$$

In deriving Eqns. 17 and 18 it was assumed that the reaction kinetics of the generation of charge carriers from excitons as the first order. This simplification may be justified as shown in the development of the possible mechanism of charge carrier generation as described in the following paragraphs.

The processes leading to the generation of electronic charge carriers can be considered in the following two ways. The first possibility is the charge separation *via* the photogenerated excitons and second possibility is the charge transfer complex

formation between excited retinal and electron acceptors or electron donors. The first pathway may be written as:

$$e + A \underset{k_b}{\overset{k_a}{\rightleftharpoons}} Ae \xrightarrow{k_c} A^- + \oplus \tag{20}$$

$$e + D \underset{k'_b}{\overset{k'_a}{\rightleftharpoons}} De \xrightarrow{k'_c} D^+ + e^- \tag{21}$$

where e is exciton, A is electron acceptor, D is electron donor, Ae and De are complexes for acceptor–exciton and donor–exciton, respectively (these complexes may be considered as surface traps), $\oplus$ is hole, e^- is electron, and k's are rate constants for respective processes. For the charge transfer complex pathway reactions may be written as:

$$R^* + A \underset{k_b}{\overset{k_a}{\rightleftharpoons}} AR^* \xrightarrow{k_c} A^- + R^+ \tag{22}$$

$$R^* + D \underset{k'_b}{\overset{k'_a}{\rightleftharpoons}} DR^* \xrightarrow{k'_c} D^+ + R^- \tag{23}$$

where R* is the excited retinal molecule.

Between these two types of pathways there are similarities. Excited species form a complex with acceptor or donor reversibly and the product formation is irreversible. Donors and acceptors are fixed at the interfaces and can be considered as fixed sites for adsorption. Excitons and excited retinals can be considered as the species colliding onto the fixed sites. It is assumed that excitons and excited retinals can interact with the adsorption sites in the fashion of a random walk. If the above assumptions are correct, then, the kinetics can be studied with the Langmuir adsorption isotherm. Before the development of the kinetic equations using the Langmuir adsorption isotherm, it is necessary to clarify the details of the present model.

Because of the uncertainty on the possible pathway between the proposed two pathways, the rest of the discussion will be focused on the exciton pathway. This selection does not create any inconvenience since the other pathway is quite similar to the former one.

It is expected that water itself can perform the role of electron acceptor as well as electron donor in the absence of better acceptors or donors. The reactions taking place at the inside surface are the summation of the reactions expressed by Eqn. 20 and 21. The observed net reaction is, then, the difference between the sum of the similar reactions occurring at the outside surface and the sum of the similar reactions at the inside surface. In the case of a symmetric system the rate of the generation of charge carriers is the same between two surfaces, thus, we should expect that potential development is negligible. Indeed, this is the case as commonly observed in this and previous work[26]. Because of the complexity in treating the total reactions as a general case, and yet the considerable generality of the single reaction, treated in the following paragraphs wil be an asymmetric case with Fe^{3+} in the inner chamber. This selection could practically fix the sign of photoresponses and enhance the magnitude, indicating such a simplified treatment is not only desirable but also required. Due to this factor of asymmetry a great simplification can be made in discussing the kinetics of the development of the electronic component.

In the simplest situation ferric ions function as the sites and excitons adsorb onto the surface sites. The reversible adsorption can be expressed by Langmuir's isotherm[32]. Let s be the fraction of surface that is covered and $(1 - s)$ the fraction that is bare. The rate of adsorption is then $k_a c\ (1 - s)$, where c is the concentration of the adsorbate (exciton) and k_a a constant shown in Eqn. 20 and the rate of desorption is $k_b s$. At equilibrium the rates are equal, or:

$$\frac{s}{1 - s} = \frac{k_a}{k_b} c = Kc \tag{24}$$

where K, equal to k_a/k_b, is a constant.

Similarly a first order rate equation can be derived for proton generation at the opposite surface of the bimolecular lipid membranes by assuming the Langmuir type adsorption of holes at the surface sites as an intermediate step. The diffusion process of holes and protons can be treated as the first order rate reactions. It is also possible to specify that all reactions are biased forwardly and backward rate constants are negligible. The reaction scheme may be expressed as the reactions of most possible reactants (Fe^{3+} as acceptors and water molecules as donors) as shown in Fig. 10.

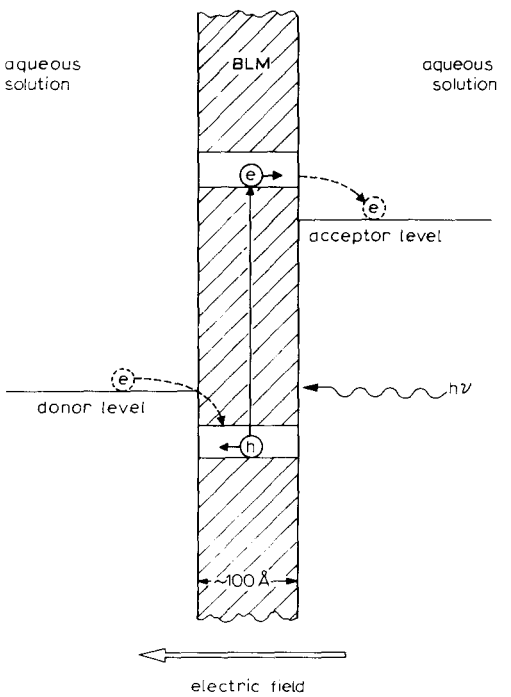


Fig. 10. A simple scheme for charge separation and energy storage across a photoactive bilayer (black) lipid membrane (BLM). The membrane is treated as an ultrathin layer of liquid crystal separating two aqueous solutions. The absorption of a photon by the pigment in the membrane causes the ejection of an electron into an excited level. In the case illustrated, the electron acceptor level and the electron donor level lie, respectively, below the conduction band and above the ground level of the membrane. Electronic charge carriers (*via* either singlet or triplet exciton dissociation) migrate in the direction of the field give rise to the observed photoelectromotive force (and photocurrent). The energy of the absorbed photon is stored in terms of redox products, since the transferred electron is at a higher level than the electron of the donor.

Sequentially, there are four major processes: (i) photon absorption leading to the generation of excitons, then holes, (ii) the diffusion of holes across the hydrocarbon layer of bimolecular lipid membranes in the same sense of the dark current, (iii) interaction of holes with water molecules leading to the proton generation, and (iv) the back flow of protons due to the newly formed proton or disturbance of the protonic concentration gradient across bimolecular lipid membranes.

Upon establishing the simplified reaction scheme and the rate equations the equilibrium concentrations of the terms shown in Eqns. 15 and 16 can now be evaluated:

$$\frac{dc_p^{i,h}}{dt} = k_1 c^e - \frac{c_p^{i,h}}{\tau_h} - D_1 c_p^{i,h} \tag{25}$$

where τ_h is the hole lifetime.
At equilibrium:

$$\frac{dc_p^{i,h}}{dt} = 0 \tag{26}$$

and

$$c_p^{i,h} = \frac{c^r k_1 \tau_e \tau_h \varepsilon I_0 e^{-\varepsilon c r_1}}{(1 + k_1\tau_e)(1 + \tau_h D_1)} \tag{27}$$

It is known that interfacial pH is determined by the equation:

$$\text{pH (surface)} = \text{pH (bulk)} + \frac{e\zeta}{kT} \tag{28}$$

where e is the electronic charge, ζ is zeta potential, k is Boltzman constant and T is absolute temperature[36]. Since zeta potential of bimolecular lipid membranes (phospholipid) is found to be small, less than 5 mV (refs. 37, 38), the correction is unnecessary. Thus, the concentration of hydrogen ions at the surface of bimolecular lipid membranes is assumed to be proportional to the bulk concentration:

$$c_d^{o,H} = A c_{\text{dark, bulk}}^{o,H} \tag{29}$$

where A is the partition coefficient of protons at hydrocarbon/water interfaces.

The formulation of the present theory was based on the fact that a pair of calomel electrodes with salt bridges can detect only the potentials developed by the membrane as a result of the separation of charge carriers. In order to use this concept the transference number of protons was experimentally determined as unity to chloride ions and the transference number of holes was also assumed to be unity. The assumption made concerning the hole transference number can be well justified by the observation that the presence of a strong electron acceptor (Fe^{3+}) can have a definite effect on determining not only the direction but also the magnitude of R1, without exception, and agrees with the sign of the potentials predicted by the assumption of the hole diffusion from the side onto which Fe^{3+} had adsorbed. This explanation is equivalent to the concept of hole injection into the hydrocarbon layer at the side of Fe^{3+} after the excitation of retinal incorporated into the hydrocarbon layer. Such a possibility was also proposed by KALLMANN AND POPE[39] to occur at semiconductor/water

interfaces. The present work strongly favors the presence of hole conduction by establishing the presence of protonic potential, which is free from the effect of other ionic species, and by demonstrating the presence of the non-protonic potential, which was proposed to be the hole potential. The fact that the non-protonic potential could not be leakage potential of other anions or cations was clearly demonstrated by the observation that the 10-fold concentration gradient of KCl and NaCl did not have any effect on the R1 and R2 components. Thus, the success of the present theory in explaining all observed facts indicates strongly that the assumption of the presence of the hole conduction in all-*trans*-retinal bimolecular lipid membranes is well justified.

ACKNOWLEDGMENT

This study was supported by Public Health Service Grant GM-14971.

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