PHOTOINITIATED MEDIATED TRANSPORT OF H_3O^+ AND/OR OH^- ACROSS GLYCEROL MONOOLEATE BILAYERS DOPED WITH MAGNESIUM OCTAETHYLPORPHYRIN

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ABSTRACT   Photogenerated magnesium octaethylporphyrin cation in glycerol monooleate bilayers is shown to mediate the transport of H_3O^+ and/or OH^-. Data from voltage clamp and open-circuit experiments are consistent with the classic Markin or Laüger carrier model. Photoinitiated currents exhibit the expected transient and steady-state behavior.

INTRODUCTION

Photocurrents across phospholipid bilayers doped with magnesium octaethylporphyrin (MgOEP) have been observed by serveral workers (Hong and Mauzerall, 1972a, b, 1974; Mauzerall and Hong, 1975; Hong, 1976; Pohl et al., 1973; Lutz et al., 1974). The underlying photoprocess is the excitation of the ground-state porphyrin (P) to an excited state, probably the triplet, P^T, which would be considerably longer lived than the excited singlet, P^* (Hopf and Whitten, 1975):

$$P \underset{k_d}{\overset{h_r}{\rightleftharpoons}} P^* \overset{k_{isc}}{\longrightarrow} P^T. \tag{1}$$

The reduction potential, $E^0_{P^+/P}$, of the ground-state couple

$$P^+ + e^- \rightleftharpoons P \tag{2}$$

is about 0.66 V vs. NHE (normal hydrogen electrode).[1] The reduction potential of the cation/triplet couple, $E^0_{P^+/P^T}$,

$$P^+ + e^- \rightleftharpoons P^T, \tag{3}$$

will be shifted negative by a voltage corresponding to the energy of the triplet state (Gouterman and Holten, 1977) which is about 1.8 eV (see Hopf and Whitten, 1978, who

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[1]Fuhrhop and Mauzerall (1968, 1969) measured $E^0_{P^+/P} = 0.66$ V in alcoholic solvents using a potentiometric titration technique. (They also noted that the MgOEP$^+$ cation was stable when the solvent contained 20% water). Subsequent measurements by Kadish and Davis (1973) in butyronitrile and dimethylsulfoxide using cyclic voltammetry yielded $E^0_{P^+/P} = 0.78$ V vs. NHE. The lower value may be due to stabilization of the charge of the cation by solvent interaction with the axial ligands of the magnesium (see Fajer et al., 1973; and Davis, et al., 1979).

tabulate triplet energies for a variety of metalloporphyrins. MgOEP is not specifically included, but it is clear that the triplet energies are all about 1.8 eV). Thus $E^0_{P^+/P^T} \simeq -1.1$ V vs. NHE. In the presence of an electron acceptor (ferricyanide [Hong and Mauzerall, 1972a,b, 1974; Mauzerall and Hong, 1975; Hong, 1976] or oxygen [Pohl et al., 1973; Lutz et al., 1974]) the triplet will be oxidized:

$$P^T + A \xrightarrow{k_4} P^+ + A^-. \tag{4}$$

As long as the reduction potential, E^0_{A/A^-}, for the A/A^- couple is more positive than ~ -0.7 V, the equilibrium for reaction 4 is virtually completely to the right. It is also important, of course, that E^0_{A/A^-} be more negative than $E^0_{P^+/P}$ so that the equilibrium constant, K_5, for the ground-state reaction

$$P + A \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} P^+ + A^- \tag{5}$$

is very small and reaction 5 effectively does not occur:

$$K_5 = \exp\left[\frac{F}{RT}(E^0_{A/A^-} - E^0_{P^+/P})\right]. \tag{6}$$

The mechanism of the photoconductance observed by Lutz et al. is not elucidated. They do, however, demonstrate that MgOEP$^+$ is photogenerated in liposomes in the presence of oxygen as an acceptor. This, coupled with the experiments and conclusions of Hong et al.,[2] supports the notion that MgOEP$^+$ is involved in the photoconductive mechanism.

It is well known that a membrane formed from glyceromonoolate (GMO) rather than from phospholipids offers a lower energy barrier to the translocation of hydrophobic cations (Szabo and Eisenman, 1973; Hladky and Haydon, 1973). If the MgOEP$^+$ cation plays an active role in the photoconductance phenomena, this role would be enhanced in GMO bilayers. In the present paper we demonstrate that the photogenerated MgOEP$^+$ cation probably acts as an ion carrier and under certain conditions selectively mediates the transport of hydrogen ion and/or hydroxide.

EXPERIMENTAL

In experiments that we describe here, our basic approach is the observation of the electrical responses of the doped bilayer following a single laser flash (duration $\sim 1 \mu s$). A schematic presentation of the experimental arrangement is shown in Fig. 1. The aqueous phases contain varying concentrations of buffer (malate or phosphate, both of which will be shown not to move across the bilayer), supporting electrolyte (1.0 M KCl except where noted) and

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[2]Hong et al. observe a displacement current affected by having the acceptor, ferricyanide, present on only one side of the bilayer. The flash is accompanied by a virtually concomitant charge separation with the electron moving from the triplet (in or on the bilayer) to the acceptor in the diffuse Gouy layer. Charge translocation across the entire bilayer does not play a major role in their observations. Our immediate interest is the clarification of the mechanism of charge translocation across a bilayer which is subjected to an applied electric field.
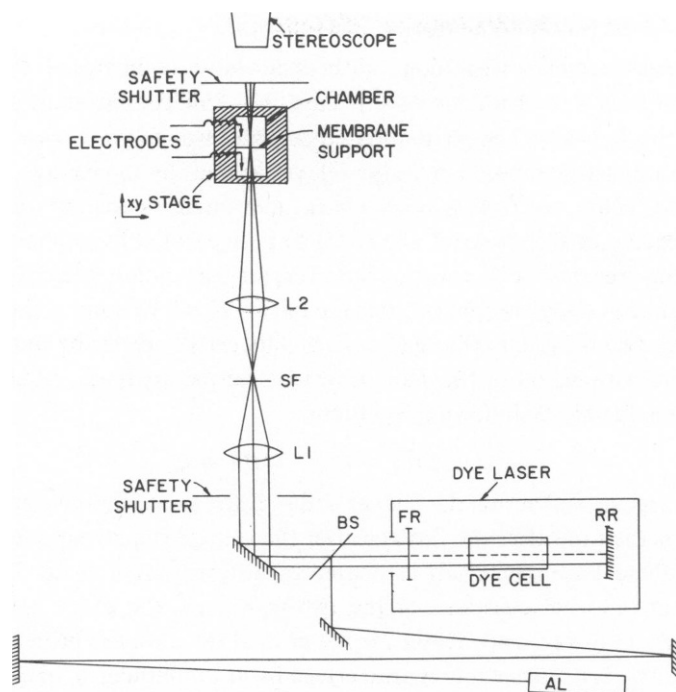
FIGURE 1 *AL*, alignment laser; *RR*, rear reflector of laser cavity; *FR*, front (partial) reflector of laser cavity; *BS*, 60% transmitting beam splitter; *LI*, 20-cm focal length lens; *SF*, pinhole spacial filter; *L2*, 10-cm focal length lens. Safety shutters are electrically interlocked to avoid accidental photolysis of stereoscope. The stereoscope is used from behind the cell to aid in membrane formation.

acceptor (methyl viologen; $E^0_{MV^{++}/MV^{+}} = -0.46$ V vs. NHE [Stombaugh et al., 1976] with or without oxygen; $E^0_{O_2/O_2^-} = -0.55$ V vs. NHE in N,N'-dimethylformamide [Magno et al, 1977]). Methyl viologen was selected for a number of reasons: it is very effective, possibly because both products of reaction 4 are positively charged, thereby favoring production of the MgOEP$^+$ cation (see Holten et al., 1978); its very negative reduction potential ensures that there is no trace of the reduced form to react with the MgOEP$^+$ and further ensures that the equilibrium of reaction 5 lies far to the left (ferricyanide fails in both these respects); preliminary experiments in which the reduced form of methyl viologen (MV$^+$) was introduced into the membrane bathing solution by reduction of MV^{++} with colloidal zinc indicated that MV$^+$ does not move across the GMO bilayer.

Two types of experiments have been carried out: voltage clamp measurements, where the observed photoresponse, proportional to the integral of the current passed, $q = \int_0^t i \, dt$, is observed as a function of time; and open circuit measurements, where voltage is observed as a function of time. Following the integrated current rather than the current offers several advantages: the charge, in which we are most interested, can be directly measured; the amplifier is not saturated during capacitive charging. The disadvantages are that evaluation of rate constants is more complicated and transients with small amplitudes and/or short time constants may be more difficult to see.

Voltage Clamp Measurements

Voltage clamp measurements were done with chemically symmetrical systems (i.e., the aqueous phases on each side of the membrane have the same composition) and with applied voltages (V_a) up to ± 0.350 V. The photoinitiated response was followed as a function of time and reflects the total charge moved across the bilayer. Generally the voltage, V_a, was applied several milliseconds before the flash to allow charge movement associated with small changes in membrane capacitance (see Benz et al., 1975) to be separated from photoinduced charge movement. In some "reversal" experiments, however, for reasons amplified in the Results and Discussion section, the voltage is applied after the flash. As will be seen, a photo response at a given V_a comprises two transients whose time constants usually differ by more than an order of magnitude. The parameters of the short transient can be analyzed by using a nonlinear least-square routine fitting the following equation:

$$q_t = (A/a)(1 - e^{-at}) + B t + q_0, \tag{7}$$

where q_t is the charge moved across the bilayer and q_0 is the capacitive charge associated with a change in V_a; t is the time after the flash or after the voltage step, whichever happened last. The currents (or fluxes) are then easily estimated by differentiation of Eq. 7. Because of the great difference in the time constants of the two transients, the exact nature of the slow transient (which is not always exponential) is not critical since we are primarily interested in the fast transient. The fast transient is characterized by its amplitude, A/a; the initial current, A; and the rate constant, a, or halflife, $0.69/a$. The charge associated with the fast transient is

$$\Delta q = A/a. \tag{8}$$

The slow transient is characterized by an initial current, B (Eq. 7). Its halflife, however, is estimated by a least-square sliding fit of a quadratic equation and differentiation. Both fluxes (mole cm^{-2} s^{-1}) and surface concentration (mole cm^{-2}) will be expressed per unit area of membrane, A_m (see Methods and Materials):

$$f = \frac{dq_t}{dt}\bigg/ (A_m \cdot F) \tag{9}$$

$$\Delta Q = \Delta q / (A_m \cdot F) \tag{10}$$

Open-Circuit Measurements

Open-circuit voltage measurements of chemically asymmetric systems (the aqueous phases on each side of the membrane have different composition) can indicate whether or not a particular ion preferentially moves across the bilayer and if that movement is photoinitiated. If the concentrations of a given ion are different on each side of the membrane and if the membrane is selectively permeable to that ion, the voltage developed across the membrane is predicted by the Nernst equation:

$$V' - V'' = (RT/z_i F) \ln (c_i''/c_i'), \tag{11}$$

where the ($'$) and ($''$) indicate a given side of the membrane, and c_i'' and c_i' are the concentrations of the permeant ion in the two phases. If there is more than one permeant ion and $|z_i|$ for all ions are the same, then the voltage is predicted by the Goldman equation

(Goldman, 1943):

$$V' - V'' = \frac{RT}{F} \ln \frac{\sum_{j+-1}^{n} P_{j+} c_{j+}'' + \sum_{j--1}^{n} P_{j-} c_{j-}'}{\sum_{j+-1}^{n} P_{j+} c_{j+}' + \sum_{j--1}^{n} P_{j-} c_{j-}''}, \tag{12}$$

where $j+$ and $j-$ subscripts indicate cationic and anionic species; P_{j+} and P_{j-} indicate the permeabilities of the ions. It is not difficult to devise experiments in which all ratios, $c_{j\neq i}''/c_{j\neq i}'$, are so close to unity that Eq. 12 can be rewritten:

$$V' - V'' = \frac{RT}{z_i F} \ln \frac{c_i'' + d}{c_i' + d}, \tag{13}$$

where d is a constant whose magnitude and sign will depend upon the permeabilities of the other (non-i) ions in the system. Thus, a voltage response of appropriate sign and magnitude can be unequivocal evidence that the ith ion is the permeant species.

METHODS AND MATERIALS

The chamber design is essentially the same as that described by Feldberg and Kissel (1975), but constructed with pyrex windows in each end. A 2-mm diameter hole drilled through a 10-mil Teflon (DuPont Instruments, Wilmington, Del.) sheet serves as the membrane support orifice. The silver wire electrodes are carefully placed so that they are not in the light path. The area of each electrode (~ 2 cm^2) is large enough so that the electrode capacitance ($>10^2 \mu F/cm^2$) can minimize short-time polarization of the electrode. In some experiments the electrodes were momentarily short-circuited by using a manually controlled microswitch.

The photolysis laser is a pulsed dye laser (model SLL 625, Candela Corp., Needham Heights, Mass.) used with rhodamine 6G dye. The output energy is about 1 J in less than a microsecond when the laser capacitor is charged to 20 kV. The wavelength of the photolyzing flash, about 590 nm with a width at half height of ~ 40 nm (Marling, et al., 1974), overlaps nicely with the 578-nm absorption band of MgOEP (Fuhrhop and Mauzerall, 1968).

A small continuous wave He-Ne laser (0.5 mW) was used to align the dye laser cavity for optimum output and to align the membrane support orifice with the laser beam. The opitcal bench (Fig. 1) is designed so that the beam of the alignment laser is colinear with the beam of the dye laser. It is important that only the bilayer (and not the torus) be irradiated (Mauzerall and Hong, 1975). Alignment of the bilayer orifice was effected by allowing the reflected beam of the alignment laser to impinge on the 10-mil Teflon barrier a few millimeters to one side of the orifice. The thin Teflon acts like a ground glass imager allowing the beam position to be located and its diameter to be crudely estimated with the stereo microscope. By forward or backward movement of the chamber (which rests on a micrometer controlled X-Y stage) the diameter of the laser beam intercepting the barrier can be adjusted. An apparent diameter of approximately two-third the diameter of the bilayer was considered optimum, although the response does not critically depend upon the apparent illuminated area (probably because of the high degree of saturation of the photo processes). Finally, the alignment beam was blocked and the chamber moved laterally so that the center of the orifice would coincide exactly with the center of the beam. With proper alignment, ~ 0.05 J per pulse of the dye laser impinged upon the bilayer (~ 2.5 J/cm^2). Precisely controlled diminution of the laser intensity was effected with neutral density filters placed directly in front of the laser.

The membrane-forming mixture comprises GMO, hexadecane, CH$_2$Cl$_2$, and MgOEP in molar ratios 3:80:100:1 and was prepared fresh for each day's experiments. 1 mg of MgOEP was dissolved in 25 mg of CH$_2$Cl$_2$ followed by addition of 0.05 ml of hexadecane containing 2.5 mg of GMO. In a few experiments the MgOEP concentration was reduced by diluting with the appropriate mixture of CH$_2$Cl$_2$, hexadecane, and GMO. Membranes were formed using the pipette technique of Szabo et al.

(1969). Voltages up to 0.20 V were applied to initiate bilayer formation. Most measurements were made within 1 min from the time that the formation of the bilayer was complete (i.e., when the bilayer was completely "black"). The bilayer was observed by using reflected red light ($\lambda > 600$ nm); this minimized inadvertent photolysis, since the lowest energy absorption band of MgOEP peaks at 578 nm.

The pH's of the solutions in each side of the chamber were measured with a glass electrode that was continually calibrated in a standard phosphate buffer (pH 7.00).

Bathing solutions contained 1.0 M KCl, up to 0.02 M methyl viologen, and varying concentrations of phosphate buffer components ($HPO_4^=$, $H_2PO_4^-$) or malate buffer components ($B^=$, HB^-, H_2B, $B^= = [-OOC-CH_2OH-CH_2-COO-]^=$). In some experiments KCl was replaced with $NaClO_4$, KPF_6, or $MgSO_4$. Solutions of KCl were freshly prepared every 3 or 4 d. Buffer stocks were prepared daily. In chemically asymmetric experiments where the compositions of the solutions on each side of the membrane were different, intermixing was minimized by blocking the orifice with an excess of the membrane-forming material.

For experiments in which oxygen was excluded, we used an air-tight plexiglass box ($11.4 \times 11.4 \times 5$ cm). Rubber septums in the top of the box allowed electrode leads and syringe needles to be introduced into each side of the chamber. A vial containing the membrane-forming mixture was placed inside the box and the Pasteur pipette (used to form the membranes) was inserted through a soft rubber septum made from a balloon or surgical glove. There is enough mobility to permit the pipette to be loaded and the membrane to be formed without removing the pipette tip from the box. The solutions in each side of the chamber were purged with solvent-saturated argon or nitrogen introduced through 18-gauge hypodermic needles. After 30 min of purging, the needles were repositioned so that a gentle stream of inert gas continuously flowed over the solutions maintaining a slight positive pressure inside the box. Open-circuit voltages were measured using a field effect transistor input follower circuit (Instrumentation Division, Brookhaven National Laboratory). The voltage clamp-current integrator has been previously described (Feldberg and Delgado, 1978). A function generator (model 175, Princeton Applied Research Corp., Princeton, N.J.) programmed the clamp potential.

An Orion model 601A pH meter and electrode model 91-02 (Orion Research Inc., Cambridge, Mass.) were used to measure pH.

Data were collected and stored with a Nicolet 1090 digital oscilloscope and NIC-283 tape coupler (Nicolet Instrument Corp., Madison, Wis.) with a Kennedy 9700 tape drive (C.J. Kennedy Co., Altadena, Calif.). The tape served both for data storage and for communication with CDC 7600 computer (Computer Data Corp., Minneapolis, Minn.). Computer generated figures were executed with a display integrated software system and plotting language (DISSPLA) package (Integrated Software Systems Corp., San Diego, Calif.).

Sequencing and timing of events (triggering of oscilloscope, integrator, voltage pulse, laser flash) were accomplished with a home-built sequencer (Instrumentation Division, Brookhaven National Laboratory).

Racemic GMO was obtained from Supelco (Supelco Inc., Bellefonte, Pa.). Practical grade hexadecane and laser grade rhodamine 6G perchlorate were obtained from Eastman Kodak (Rochester, N.Y.). Methyl viologen chloride (Aldrich Chemical Co., Inc., Milwaukee, Wis.) was recrystallized from methanol. Elemental analysis indicated 4 waters of crystallization and a mol w of ~329. Methylene chloride (Fisher Scientific Co., Pittsburgh, Pa.), was refluxed with CaH_2 and distilled over 4A molecular sieves. The MgOEP was a gift from J. Fajer (Brookhaven National Laboratory). All other chemicals were analytical grade. High-purity deionized (Milli-Q) water was used throughout (Millipore Corp., Bedford, Mass.).

RESULTS AND DISCUSSION

Voltage Clamp Experiment

A voltage clamp experiment (see Fig. 2 a for the sequencing protocol) with phosphate or malate buffer and methyl viologen as the acceptor exhibits a photoinitiated response
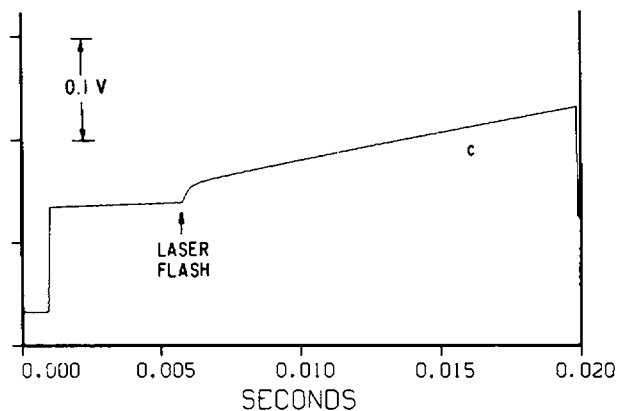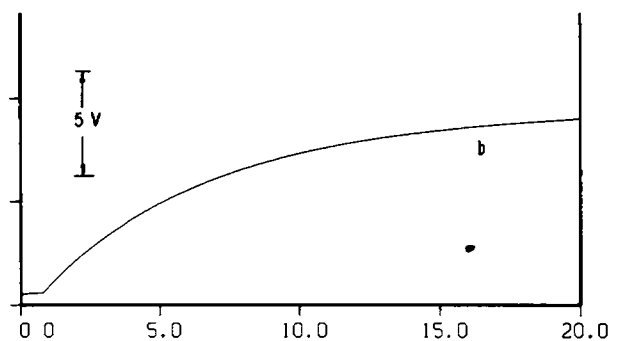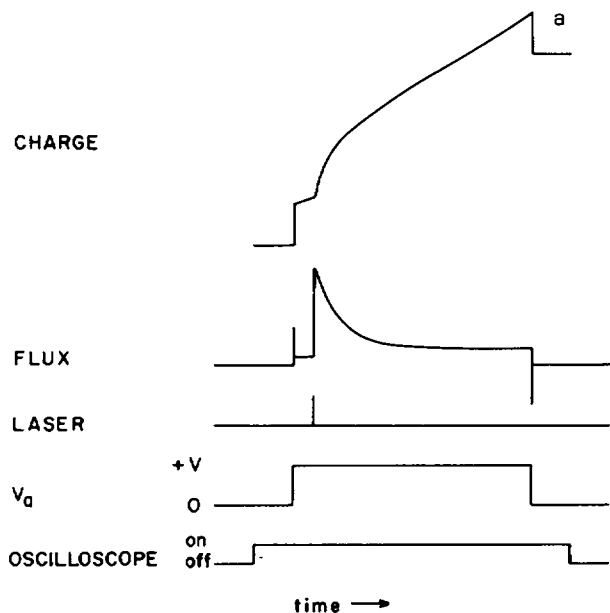
FIGURE 2 (a) Sequence of events in voltage clamp experiment (V_a – 0.300 V). Typical record showing (b) slow transient, and (c) fast transient when both solutions are 1.0 M KCl, 0.0079 M MV^{2+}, 0.010 M malic acid/malate pH – 5.0. Integrating capacitor is 4.8×10^{-8} F. Membrane area is 0.026 cm^2.
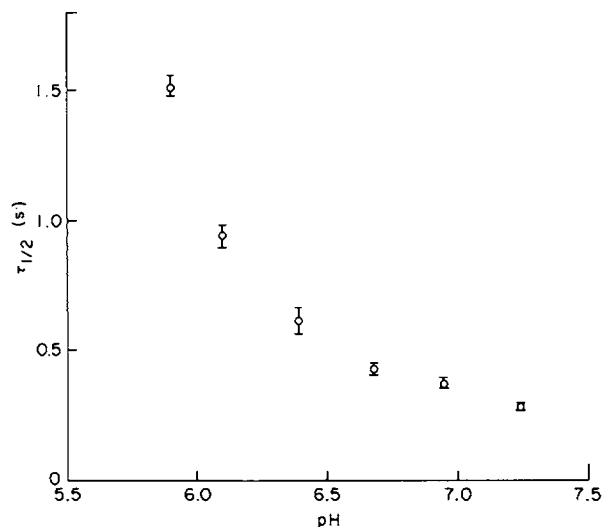
FIGURE 3 Halflife of slow transient vs. pH in phosphate buffer. Both solutions are 1.0 M KCl, 0.0079 M MV^{2+}, 0.010 M $H_2PO_4^-/HPO_4^{2-}$.

comprising two transients: a slow, approximately exponential transient (Fig. 2 *b*) with a halflife of the order of seconds and a fast, exponential transient (Fig. 2 *c*) with a halflife of the order of milliseconds. The halflife of the slow transient is virtually independent of V_a but does shorten with increasing pH (Fig. 3). At a given pH its amplitude increases with an increase in V_a (Fig. 4). The halflife of the fast transient, however, decreases with increasing V_a; the rate constant (obtained from a fit of Eq. 7) increases with V_a (Fig. 5). The amplitude, A/a (Eq. 7)
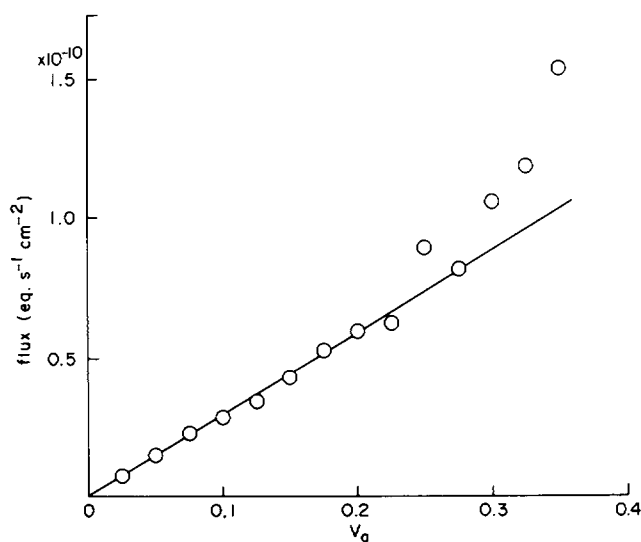
FIGURE 4 Initial flux (not corrected for background) of slow transient as a function of voltage under voltage clamp conditions. 1.0 M KCl, 0.0079 M MV^{2+}, 0.010 M malic acid/malate, pH = 5.0.
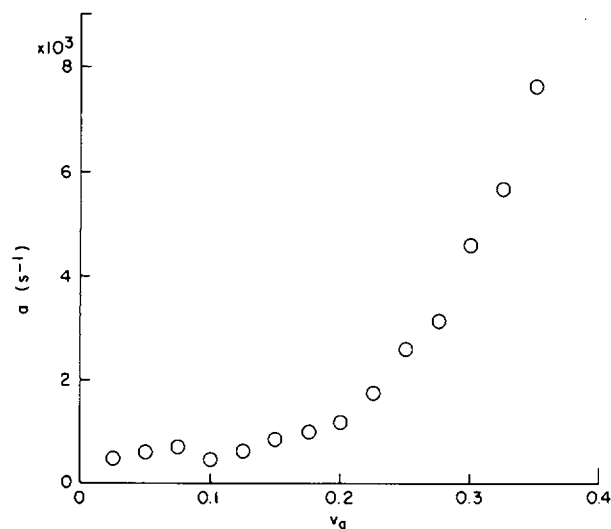
FIGURE 5   Rate constant of fast transient vs. applied voltage (Eq. 7). Solutions same as for Fig. 2.

increases with V_a, and at pH <5 clearly approaches a limiting value (Fig. 6). All the photoinitiated responses can be eliminated either by removal of the MgOEP from the membrane-forming mixture or by removal of the acceptor(s) from the aqueous phases (oxygen is removed by deaeration). With no methyl viologen and air-saturated solutions, there is a diminished but significant response. The removal of oxygen, however, from solutions containing methyl viologen does not significantly alter the response.

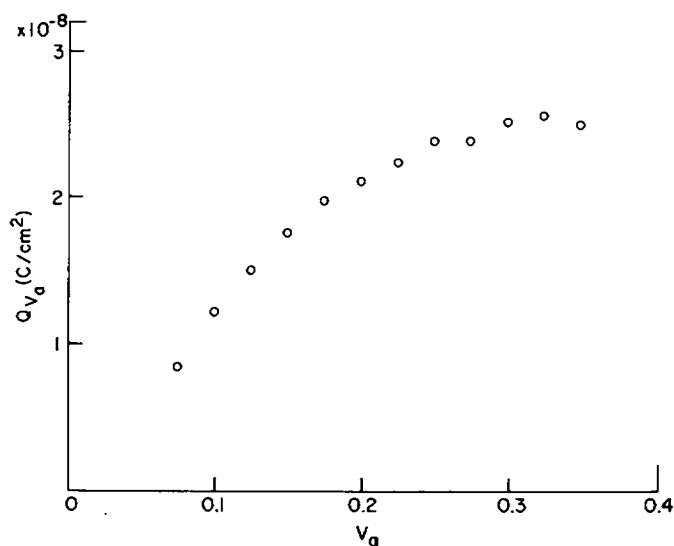Experiments were carried out to characterize the effects of varying the concentrations of

FIGURE 6   Amount of charge of fast transient translocated as a function of applied voltage. Both solutions are 0.92 M KCl, 0.020 M MV^{2+}, 0.010 M malic acid/malate, pH – 4.44. $Q_{V_a} - A/A_m a$ (Eq. 7).

MgOEP in the membrane-forming mixture, methyl viologen (MV^{++}) in the aqueous phases, and of varying the light intensity. At a fixed concentration of MV^{++} and fixed light intensity, the photoinitiated electrical response seemed proportional to the concentration of MgOEP in the membrane-forming mixture (Fig. 7), although the responses at high voltages for the highest concentrations were unaccountably low.

With a fixed concentration of MgOEP and fixed light intensity, the dependence of the response upon the concentration of MV^{++} was sublinear. We did not detail this relationship and decided upon 0.01 M MV^{++} as adequate.

With fixed concentrations of MgOEP and MV^{++} we noted that the light intensity could be diminished by a factor of five with virtually no diminution in response, indicating that the photo process is saturated.

These data suggest several preliminary conclusions: (*a*) MgOEP in the bilayer, an acceptor in the aqueous phases, and a photolysing flash are all required to initiate an electrical response. (*b*) Methyl viologen seems to be a more effective acceptor than oxygen, perhaps simply because it is present at considerably higher concentrations. The role of oxygen is both subtle and complex (see Lutz et al., 1974) and not well understood. (*c*) The short transient is well described by Eq. 7, suggesting that it reflects a first-order process. The amplitude of the short transient appears to limit (at low pH) as V_a increases. This limit, $V_{l,lim}$, probably corresponds to the translocation of all the photogenerated $MgOEP^+$ from one side of the bilayer to the other and thus serves as a means of estimating the surface concentration (P_0^+) of the cation:

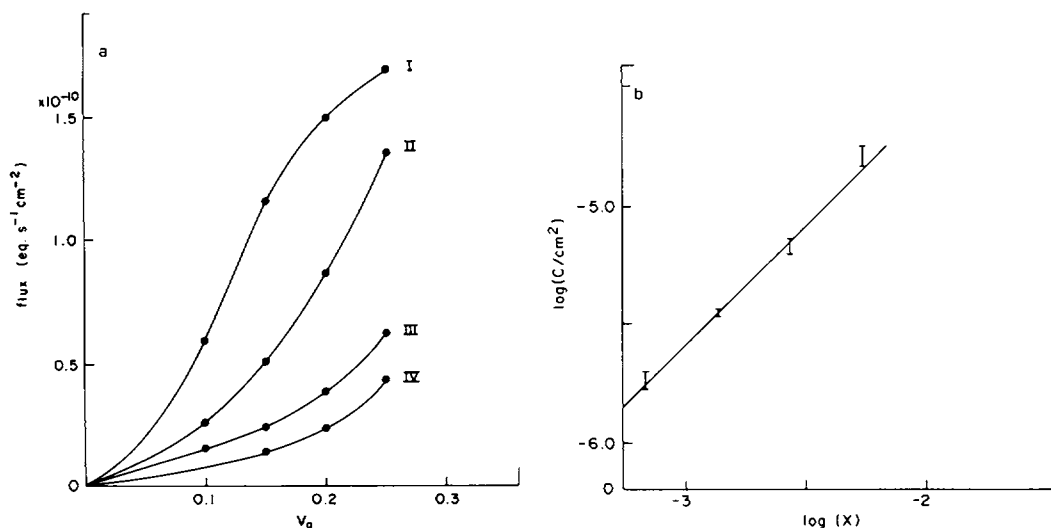$$P_0^+ = V_{l,lim} C_l / (A_m F) = mol/cm^2, \qquad (14)$$

FIGURE 7 (*a*) Initial flux of slow transient as a function of voltage for various porphyrin concentrations in membrane-forming material. I, standard concentration (see methods). II, a 1:1 dilution of I with membrane-forming material prepared in identical fashion except for addition of porphyrin. III and IV are successive 1:1 dilutions. (*b*) Log of amount of charge transferred during slow transient after 8 s vs. log of mole fraction (*X*) of MgOEP in the membrane-forming mixture. Line is unity slope. Conditions same as for Fig. 3; pH – 6.25, V_a – 0.100 V.

(where C_i is the value of the integrating capacitor, A_m is the area of the membrane, F is the faraday). Since the photo process appears to be saturated, it may be reasonable to assume that nearly all of the neutral porphyrin (P_0) is oxidized to P^+. Then

$$P_0 = P_0^+ = 2.7 \times 10^{-13} \text{ mol/cm}^2. \tag{15}$$

If we assume a membrane thickness of about 3.0 nm, this surface concentration corresponds to about 1.8×10^{-6} mol/cm^3 in the bilayer. This is about 10-fold smaller than the concentration in the membrane-forming mixture and consistent with the observations of Cherry et al., (1971) who were able to use optical absorption techniques to directly measure concentrations of chlorophyll in phospholipid bilayers. The long transient can be shown not to be the simple translocation of the porphyrin cation. The measured voltage across the integrating capacitor (Fig. 2 c) is so large that one would deduce an intermolecular distance for the adsorbed cations that is less than the diameter of a porphyrin molecule. (d) The ion flux that exists during the course of the long transient must involve the transport of some ion in the aqueous phase, since it cannot reasonably be a simple translocation of the porphyrin cation. Furthermore, the MgOEP$^+$ cation must actively mediate this flux. The decay of this flux must then reflect the disappearance of the porphyrin cation from the bilayer by some chemical or physical (e.g., desorption) process. The approximately exponential decay of the flux would seem to preclude diffusion control by some species in the aqueous phase. This conclusion is consistent with the observation that the time constant of the slow transient is independent of V_a, while the amplitude increases with increasing V_a. This is easily shown if we assume that the cation disappears by some first-order process with rate constant k_x. Then

$$P^+ = P_0^+ \, e^{-k_x t}. \tag{16}$$

If the ionic flux is related to the cation concentration by some voltage dependent rate constant, k_v, then

$$f = k_v P^+ = k_v P_0^+ e^{-k_x t} \tag{17}$$

and

$$(df/dt) = -k_x k_v N_0 e^{-k_x t} = -k_x f. \tag{18}$$

Therefore

$$f = f_0 e^{-k_x t} \tag{19}$$

Clearly, the time constant will be independent of k_v and therefore of V_a, while the integrated amplitude

$$\int_0^\infty f \, dt = \frac{k_v}{k_x} P_0^+ \tag{20}$$

will increase with V_a.

If it is true that the short term transient reflects the translocation of the porphyrin cation, a second voltage perturbation in which the sign of V_a is reversed ($V_a \rightarrow -V_a$) should exhibit a transient with the same halflife but twice the amplitude, i.e., $Q_{-V_a}/Q_{V_a} = 2.0$. The argument for this is quite simple. The first response initiates from a membrane that has the same

number of cations on each side which (we assume) are in chemical equilibrium with the environment:

$$(P^+)'_{t=0} = (P^+)''_{t=0} = P_0^+. \tag{21}$$

When the fast transient is complete

$$(P^+)'_{V_a} = P_0^+ + \Delta P^+ \tag{22}$$

$$(P^+)''_{V_a} = P_0^+ - \Delta P^+ \tag{23}$$

and the amplitude of the transient corresponds to the translocation of ΔP^+ mol of MgOEP$^+$. After reversal and after the fast transient is complete then

$$(P^+)'_{-V_a} = P_0^+ - \Delta P^+ = (P^+)'_{V_a} - 2\Delta P^+ \tag{24}$$

and

$$(P^+)''_{-V_a} = P_0^+ + \Delta P^+ = (P^+)''_{V_a} + 2\Delta P^+ \tag{25}$$

and clearly $2\Delta P^+$ mol are translocated. Thus the ratio of the amplitudes should be 2.0.

Several reversal experiments were carried out at pH 4.8 with malate buffer. The sequencing protocol is shown in Fig. 8 a. If the flash occurs after the application of V_a, we noted that the ratio Q_{-V_a}/Q_{V_a} was about 1.5, significantly less than the predicted value of 2.0. Thus we introduced an additional experimental variable, $\tau_{V_a} - \tau_F$, the time delay between the flash and the application of voltage, V_a. A negative value indicates that the voltage was applied first, followed by the flash; a positive value indicates that the voltage was applied after the flash. The time of the reversal, τ_{-V_a}, was 0.02 s greater than τ_{V_a}. The amplitudes were evaluated by fitting Eq. 7 to the data and the ratio, Q_{-V_a}/Q_{V_a} was plotted vs. $\tau_{V_a} - \tau_F$ (Fig. 8 b). The ratio clearly approaches 2.0 with longer delay times. We also noted (Fig. 8 c) that the increase in the ratio is due to a decrease in Q_{V_a} with increasing delay time. Q_{-V_a} remains nearly constant. There is a small decrease of both Q_{V_a} and Q_{-V_a} with longer delay times which is consistent with the previously observed decay of the slow transient.
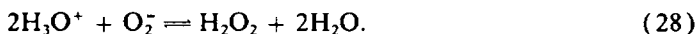
The reversal experiment seems to support the notion that the prophyrin cation is translocated in the early stages of the response. At this time, we can only surmise that the decay of Q_{V_a} with increased delay time (Fig. 8 b) may be due to back reaction of P^+ with reduced methyl viologen (MV$^+$) or with the superoxide anion (O$_2^-$). The latter could arise by (Patterson et al., 1977):

$$\mathrm{MV^+ + O_2 \rightarrow MV^{++} + O_2^-} \tag{26}$$

and

$$\mathrm{MV^+ + O_2^- \rightarrow MV^{++} + O_2^-} \tag{27}$$
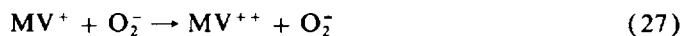
ultimately forming hydrogen peroxide

$$\mathrm{2H_3O^+ + O_2^- \rightleftharpoons H_2O_2 + 2H_2O.} \tag{28}$$

The O$_2^-$ can also disproportionate to form O$_2$ and H$_2$O$_2$ (Bielski and Allen, 1977). We have ascertained that H$_2$O$_2$ does not reduce the porphyrin cation (addition of H$_2$O$_2$ to the aqueous phase did not significantly alter the halflife of the slow transient). Thus this mechanism provides a route for inhibition of the back reaction (see reaction 5).
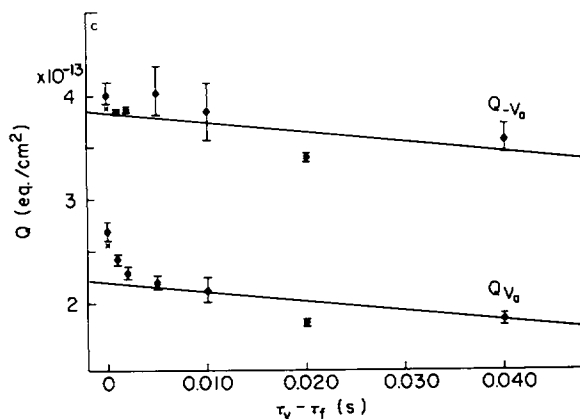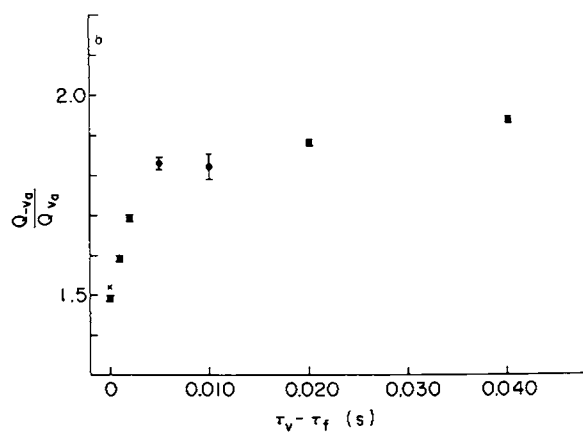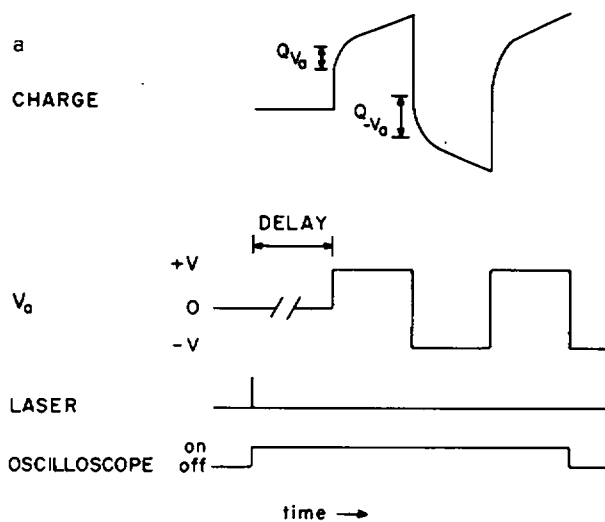
FIGURE 8 (a) Sequence of events for "delay" experiments. (b) Ratios of initial amount of charge transferred to the amount transferred upon voltage reversal as a function of delay. Both bathing solutions are 1.0 M KCl, 0.0079 M MV^{2+}, 0.010 M malic acid/malate, pH — 4.8. (c) Amounts of charge transferred vs. delay. Symbol x indicates that application of V_a preceded the flash.

We return now to our conclusion that some ion in the aqueous phase is being transported across the bilayer during the slow transient. Several voltage clamp experiments were carried out using methyl viologen as acceptor and a variety of unbuffered electrolytes: KCl, $NaClO_4$, KPF_6, and $MgSO_4$. With KCl, the concentrations were varied from 1 M to 0.001 M. In all cases, significant photoinitiated electrical responses were observed that were qualitatively similar to those we have described. We concluded that none of the ions associated with these salts was being transported during the slow transient. We suspected that hydrogen ion and/or hydroxide might be involved and designed several open-circuit experiments to verify this hypothesis.

Open Circuit Measurements

SYMMETRIC PH, ASYMMETRIC BUFFER   With KCl as the major electrolyte, phosphate or malate buffer, and methyl viologen, several chemically asymmetric experiments were carried out in which the pH's of the solutions on each side of the membrane were the same but
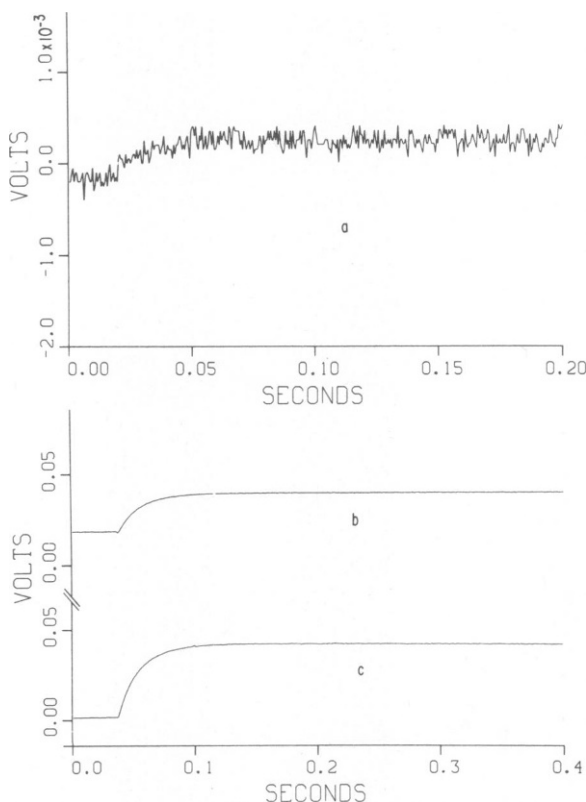

FIGURE 9   Open-circuit measurements. (a) Symmetric pH, asymmetric phosphate buffer. Left bathing solution: 1.0 M KCl, 0.0079 M MV^{2+}, 0.01 M $H_2PO_4^-/HPO_4^{2-}$, pH – 6.55. Right bathing solution: 1.0 M KCl, 0.0079 M MV^{2+}, 0.001 M $H_2PO_4^-/HPO_4^{2-}$, pH – 6.45. (b) Asymmetric pH. Left pH – 6.00, right pH – 6.85. Both bathing solutions are 1.0 M KCl, 0.0079 M MV^{2+}, and 0.010 M $H_2PO_4^-/HPO_4^{2-}$. (c) Identical conditions to (b) except that the electrodes were momentarily short-circuited just before electrolysis.

the concentration of buffer components were different. Typically the dark-potential (the open-circuit potential across the membrane before exposure to light) was very close to zero and no change in the open-circuit potential was observed after the flash (Fig. 9 a). A reasonable conclusion (Eq. 13) is that no buffer components are moving across the bilayer.

ASYMMETRIC PH   When the pH's on each side of the membrane were different, however, a dark-potential was observed and a significant photoinitiated response was also observed (Fig. 9 b). The sign of the dark and of the photoinitiated response is consistent with hydroxide moving from the high pH side to the low and/or with hydrogen ion moving from the low pH side to the high. The halflife for this response is about 5–10 ms. If the electrodes were momentarily short-circuited, several seconds were required to reestablish the dark-potential (Fig. 10). If the electrodes were momentarily short-circuited just before the flash, the photoinitiated response (Fig. 9 c) reestablished the same voltage that was reached when starting from equilibrium, and does so with the same short halflife (5–10 ms.). A plot of the maximum amplitude of the photoinitiated responses vs. the difference in pH between the two aqueous phases is shown in Fig. 11. The slope is 0.053 V/pH, indicating that hydrogen ion and/or hydroxide are quite selectively transported across the membrane after the flash (Eq. 13). Perfect selectivity ($d = 0$, Eq. 13) would give a slope of 0.059 V/pH.

We also attempted experiments with no buffers in which pH was adjusted (poorly) by judicious additions of HCl or KOH. The responses were much smaller, irreproducible, and occasionally biphasic.

Several conclusions were reached on the basis of these experiments. (a) Phosphate and malate buffer components do not move across the bilayer. (b) Hydrogen ion and/or hydroxide are selectively (almost) transported across the membrane by a photoinitiated mechanism most probably involving the MgOEP$^+$ cation. (c) The buffers, in addition to maintaining constant pH and minimizing concentration polarization in the aqueous surface boundary regions, may also play a role in a generalized acid-base catalysis of the transport mechanism as evidenced by the poor responses in absence of buffer. We shall amplify this in the next section.
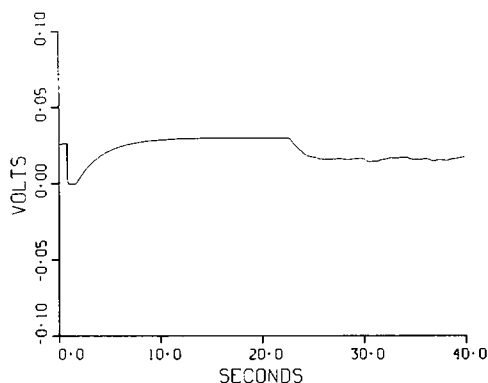

FIGURE 10   Asymmetric pH. "Dark" potential momentarily shorted to zero followed by reestablishment of dark potential. Potential instability beginning at 22 s is typical; hence, photolysis experiments were performed as soon as possible after completion of bilayer formation (<10 s). Conditions same as for Fig. 9 b.
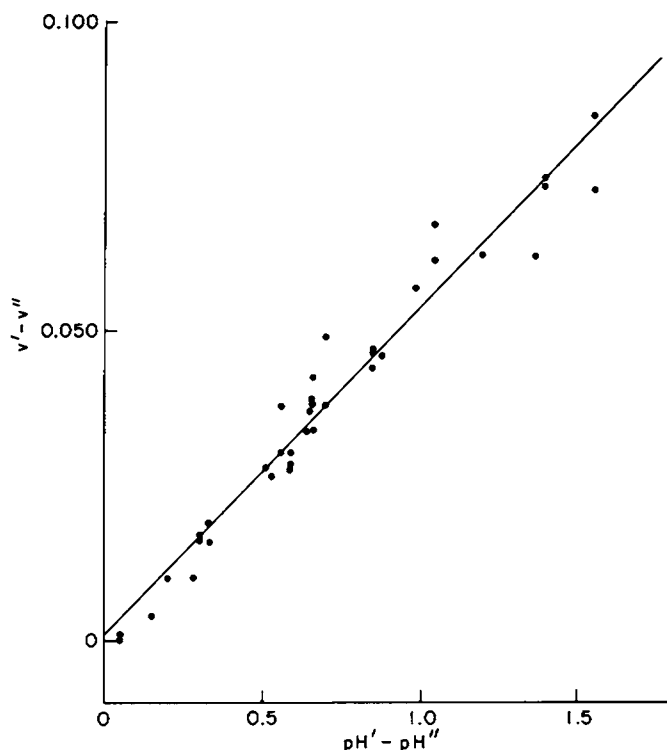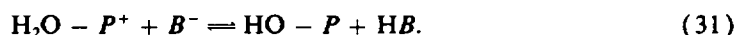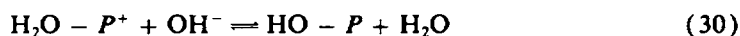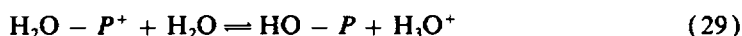
FIGURE 11   Photo voltages observed under open-circuit conditions as a function of pH gradient. Both bathing solutions are 1.0 M KCl, 0.0079 M MV^{2+}. The pH range is from 5.5 to 7.2. Phosphate buffer concentrations ranged from 0.001 to 0.010 M on each side of the membrane.

CONCLUSIONS

The experimental results confirm the hypothesis that a light flash impinging upon a bilayer doped with MgOEP initiates the transport of hydrogen ion and/or hydroxide. The fact that no photoinitiated fluxes are observed when there is no electron acceptor in the aqueous phases suggests that the photogenerated cation is an active participant in the transport process. The following reactions between the membrane bound photogenerated $MgOEP^+$ cation and the relevant aqueous components (hydrogen ion, hydroxide, and buffer) are consistent with our observations:

$$H_2O - P^+ + H_2O \rightleftharpoons HO - P + H_3O^+ \qquad (29)$$

$$H_2O - P^+ + OH^- \rightleftharpoons HO - P + H_2O \qquad (30)$$

$$H_2O - P^+ + B^- \rightleftharpoons HO - P + HB. \qquad (31)$$

We have no direct evidence for the bonded H_2O, although it is difficult to introduce the catalytic buffer dependence (reaction 31) without a labile proton. There is ample evidence that there is interaction of water with the magnesium of chlorophyll a or bacteriochlorophyll a (Katz et al, 1978) and a number of studies of solvent and electrolyte effects on the reduction
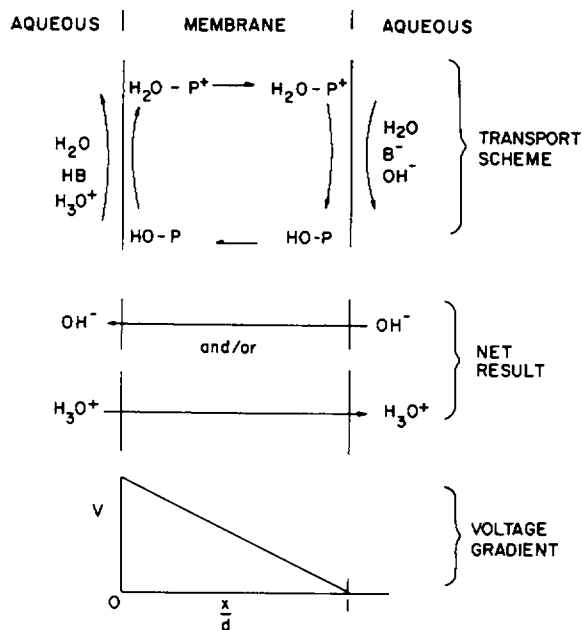
FIGURE 12 Schematic of proposed transport model for the mediated transport of H_3O^+ and/or OH^- across the membrane.

potentials of various metalloporphyrins (Fajer et al., 1973; Davis et al., 1979) further support the contention.

We surmise the transport mechanism depicted in Fig. 12. This mechanism is precisely analogous to the carrier mechanism originally described by Markin et al. (1969) which has since proved invaluable in characterizing carrier transport in lipid bilayers. In our case the net result is the transport of hydrogen ion in one direction or hydroxide in the other, depending upon how one prefers to view it. The general characteristics of the time dependence of photoinitiated voltage-clamp responses are met. What we have referred to as the slow transient reflects a steady-state flux; the reason it is not constant is due to the disappearance of $MgOEP^+$ from the membrane. The slope of the slow transient at $t=0$ would correspond to the "true" steady-state flux. The fast transient would be a manifestation of the relaxation phenomena that are predicted for this type of mechanism (Stark et al., 1971). In principle, there may be as many as three relaxations: the two predicted by Stark et al., and a third due to the fact that the system is not initially at equilibrium, since the cation may be formed faster than equilibration of reactions 29–31. With a delay between the flash and the application of V_a this third relaxation can be eliminated. Since the fast transient is described well by a single exponential decay we infer that either reactions 29–31 or the rate constant controlling the back diffusion of the neutral HO–P (Fig. 12) is rate controlling. The details of the transport mechanism will be the subject of a future publication.

There are some interesting implications of the photoinitiated transport. Most evident is that the system can act as a photoamplifier in the sense that one quantum of absorbed light can facilitate many ion translocations. Analogies to the visual receptor are tantalizing but far

fetched. The early receptor potential (ERP) of the visual receptor is most likely due to a light-induced oriented dipole in the bilayer (see Hong, 1977 for a concise review of visual receptor phenomena and for references) rather than due to an asymmetric electron transfer process of the type characterized by Hong et al. (footnote 2). It has been suggested that the ERP may be a manifestation of a gating phenomenon for the late receptor potential (LRP) and in that sense is similar to the phenomena that we have described. Another interesting possibility is to use a redox gradient and light to effect a pH gradient. With an electron acceptor on one side of the membrane and an electron donor on the other, a constant light flux will cause hydroxide to move from the acceptor side to the donor side and electrons to move in the opposite direction. It is not a "pure" light energy powered pump, but rather a light assisted pump tapping the redox energy of the electron donor and the acceptor.

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