PHOTOINITIATED ION MOVEMENTS IN BILAYER MEMBRANES CONTAINING MAGNESIUM OCTAETHYLPORPHYRIN

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ABSTRACT A photocurrent produced by planar lipid bilayers containing Mg-octaethylporphyrin in the presence of oxygen has been investigated to determine if the current is due to movement of the $MgOEP^+$ ion in the bilayer. Photoexcitation of the MgOEP is known to produce $MgOEP^+$ in the bilayer when an electron acceptor is present. However, the aqueous electron acceptors ferricynanide and methyl viologen (MV^{+2}) have opposite effects on the photocurrent. Ferricynanide decreases the photo current, even in the presence of oxygen, whereas methyl viologen increases the photocurrent, but only when oxygen is present. We attribute most of the photocurrent to the movement of superoxide anion. The difference in effect between ferricyanide and methyl viologen is attributed to (*a*) the different rates of reduction of O_2 by reduced MV^+ (fast) vs. ferrocyanide (slow) and (*b*) the known competition between ferricyanide and oxygen as the acceptor for the photoexcited porphyrin. It is inferred that most of the MgOEP is localized in the polar region of the lipid bilayer. Addition of ferrocyanide to the aqueous phase on one side of the bilayer, to trap $MgOEP^+$ produced on the other side by MV^{+2}, fails to increase the lifetime of the photovoltage. With a pH gradient across the bilayer, we observed only 5% of the photovoltage expected for the selective transport of H^+ or OH^- by $MgOEP^+$. Thus, these measurements set the lower limit for the cross bilayer transit time of $MgOEP^+$ or its charge in the range of 0.1–0.5 s.

INTRODUCTION

The pigmented planar lipid bilayer is a good system for the study of photoinitiated electron transfer reactions in a membrane environment (see Tien, 1976, and Hong, 1980 for reviews). They are especially useful in the study of interfacial charge transfer events. When an electron acceptor is present, illumination of a porphyrin containing bilayer results in oxidation of the porphyrin and reduction of the acceptor. If the electron acceptor is limited to the aqueous phase by ionic charge, electron transfer occurs across the bilayer-water interface. Such charge separation has been observed with chlorophylls or magnesium porphyrins with a varity of electron acceptors (Ilani et al., 1981). This interfacial reaction can be measured either as an open circuit voltage or as a voltage clamped current. For a linear system, these are equivalent and the choice is one of convenience or of signal-to-noise limitations (Hong and Mauzerall, 1974; Hong, 1976; Huebner et al., 1984).

The question of the movement of the oxidized pigment (P^+) in the membrane is still open. Lutz et al. (1974) concluded from the observation of a photocurrent in the absence of any added aqueous electron acceptors that oxygen, which is not limited to the aqueous phase, acts as an electron acceptor for Mg-octaethylporphyrin (MgOEP). They suggested that movement of the porphyrin cation radical (P^+) in the membrane was responsible for the observed photocurrent. Further support for P^+ movement was provided by evidence for $MgOEP^+$ acting as a carrier for H^+ (or OH^-) movement across glycerol-monooleiate bilayers (Young and Feldberg, 1979). However, it is also argued that "photo-conduction" across lipid bilayers occurs by electron hopping between pigment molecules (Feldberg, et al., 1981; Ford and Tollin, 1983).

Here we have undertaken to determine if the movement of P^+ is responsible for the photocurrent observed by Lutz et al. (1974) with MgOEP containing membranes. Measurements have been made of the applied electric field dependence of membrane photovoltage rather than of the photocurrent directly to enhance the signal-to-noise ratio. We confirm that photovoltages which are membrane voltage dependent, i.e. photocurrents, are produced by MgOEP containing membranes. However, we conclude that the carriers of the charge are most likely O_2^- or charged species derived from O_2^- and impurities in the bilayer.

MATERIALS AND METHODS

Lipid Bilayers

The membranes, pigment, acceptors, and methodology are as described before (Ilani and Mauzerall, 1981; Ilani et al., 1985). The MgOEP was dissolved to a concentration of 4 mM in a decane solution of 3% L-α-lecithin (Avanti Polar-Lipids) with 0.8% cholesterol (wt/vol). The bilayer was formed by the brush method across a 1.6-mm hole in a Teflon partition (Mueller et al., 1963). The aqueous solution was 0.1M NaCl,

0.01 M KPO_4 buffer, pH 6.8. Concentrated solutions of ferri/ferrocyanide, methyl viologen (Aldrich), glucose oxidase (US Biochemical), catalase (Sigma Chemical Co.,) and/or glucose were added to one or both aqueous sides of the cell to the desired concentration. The additions of glucose oxidase to 0.05 mg/ml, catalase to 0.025 mg/ml, and glucose to 10 mM were used to reduce the oxygen concentration to $<10^{-6}$ M, as determined by measurements of triplet lifetimes by delayed luminescence (Ilani et al., 1985).

Electrical Measurements

The membrane voltage response to a 0.3-μs FWHM, 1–10 mj, 590 nm laser pulse (flash lamp pumped dye laser with Rhodamine 6-G dye) was measured with saturated calomel electrodes (Beckman). A conical light pipe made of glass with a high index of refraction (1.693) was used to guide the light to the center of the bilayer and helped avoid illuminating the thick edge of the bilayer (Liu and Mauzerall, 1985). The electrodes were connected to a Tektronics 3A7 differential comparator amplifier and the amplified voltage digitized by a Biomation 805 transient recorder. The digitized data was either read out on a strip chart recorder or transferred to an Osborne 1 (Osborne Computer) for storage on floppy-disk and then plotted out with an HP 7225B plotter.

The thickness of the membrane was determined by measuring its capacitance (C_m) from the response of the membrane to a square wave voltage applied to one electrode and the amplifier input impedance set at 10^6 ohm. Since this impedance is less than that of the membrane, $R_m \sim 10^8$ ohm, the observed relaxation time constant is equal to $10^6 * C_m$. C_m calculated from the time constant was 5 ± 2 nF, which is 0.25 ± 0.10 μF cm^{-2}. A DC current was observed during the voltage pulse when the resistance of the membrane or cell fell below $\sim 10^7$ ohm. Some samples of MV^{+2} decreased the membrane resistance, but this was the only difference observed when these apparently impure samples of MV^{+2} were used. Such membranes were discarded.

The photovoltage was measured with the differential amplifier input impedance set at 10^{10} ohm. The membrane polarization was adjusted with a high impedance DC current source (a Hg cell with a potentiometer in series with a 10^9 ohm resistor) connected in parallel with the electrodes. When acceptor was added to only one side, a positive polarization voltage indicates that the acceptor side was made positive. The membrane and voltage source resistances act as a voltage divider to determine the membrane potential and their parallel combination determines the R of the RC time constant. Since the impedance of this measuring system is greater than that of the membrane by a factor of about 10, the relaxation time constant is largely determined by the membrane RC. The system RC time constant, under the instrumental conditions for photovoltage measurements, was determined by following the membrane voltage change when an applied DC voltage was switched on or off. It was observed that a considerable (>60 mV) voltage could form across the membrane because of leakage currents from the high impedance amplifier. Such effects can introduce spurious photovoltages and can be avoided by careful balancing of the differential amplifier and measurement of the dark voltage across the membrane.

RESULTS

Here open circuit voltages across MgOEP containing membranes have been measured. When the membrane was polarized before the laser pulse, photodepolarization of the membrane was observed, even though the only electron acceptor present was oxygen. The voltage change observed with a membrane potential of −20 mV is shown in Fig. 1. The maximum depolarization observed was linearly dependent on the applied voltage over the range examined (Fig. 2). The time at which maximum depolarization occurred was independent of the applied voltage but varied with the membrane preparation from day to day. The depolarization can be fit to an exponential rise followed by an exponential decay equal to the system RC time constant. Thus the time of the maximum is related to the difference in these two rates. The half risetime was usually 100 ms ± 10%, but has varied from 10 to 120 ms.

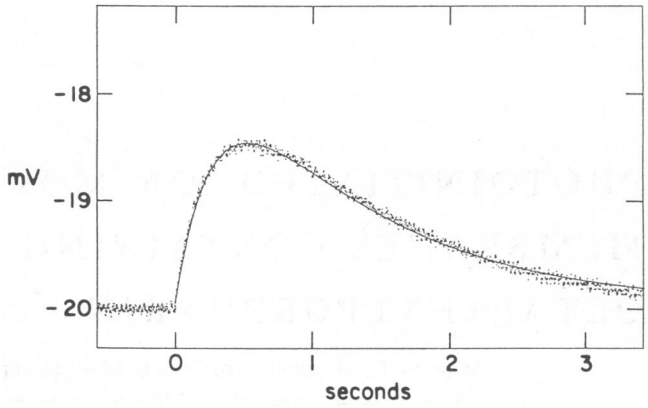


FIGURE 1 Membrane voltage is plotted vs. time. The short flash of light occurred at the time labeled zero. The MgOEP containing membrane separated aqueous buffer alone, i.e. no other additions were made. The amplifier input impedance ($1/G_v$) was $\sim 10^{10}$ ohms and the DC voltage source used to control the membrane voltage before the flash (V_b) (negative 20 mV) was in series with $1/G_b$ (10^9 ohms). The solid line is the integrated Eq. 1 with the following parameters: $C = 5$ nF, $G_m = 10^{-8}$ mho, $G_p = 2 \times 10^9$ mho, $\tau = 0.3$ s. Similar measurements over longer times showed that the photovoltage returned to the baseline within 2.5 s. The intensity of the change observed after the flash is dependent on the flash intensity which was not saturating in these results. Note that the noise level is <100 μV.

A simple interpretation for these apparent photovoltages, which are linearly dependent on the membrane potential, is that they result from photoproduced ion movements and therefore are currents or displacement currents. A voltage change of 1 mV in 0.1 s across C_m (~5 nF) represents a current of 50 pA ($I = C^* dV/dT$). Direct measurements using the voltage clamp method (Hong and

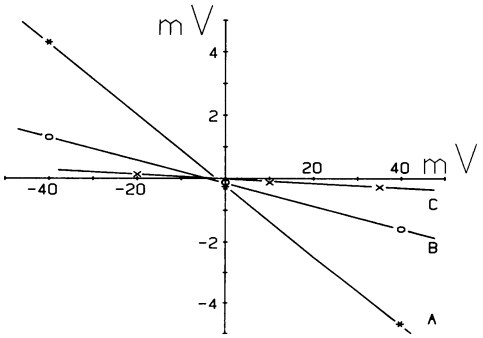
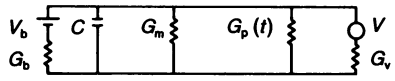


FIGURE 2 The maximum voltage change observed, as shown in Fig. 1, is plotted vs. the membrane voltage before the flash. (*A*) aqueous buffer on both sides with no other additions; (*B*) with glucose oxidase, catalase and glucose added to the aqueous buffer on both sides of the membrane; (*C*) with 5 mM ferricyanide in the aqueous buffer on both sides of the membrane. The data for *A* and *B* were obtained with the same membrane. In *B* and *C* the maximum was observed to form with the instrumental risetime, 1 μs. The photocurrent in *C* is 10% that in *A*.

Mauzerall, 1976) have shown such photocurrents but with a lower signal-to-noise ratio.

An equivalent circuit description of this interpretation consists of the parallel membrane capacitance and conductance charged with a high impedance current source and a parallel photoconductance. A parallel and separate contribution of the photochannel was shown by varying the membrane conductance externally and observing a constant photocurrent (Hong and Maurzerall, 1972). The photoproduced parallel conductance is time dependent,



SCHEME I

where V_b is the battery voltage, G_b is the voltage source limiting conductance, V and G_v represent the high impedance, 10^{10} ohm, differential amplifier, C is the membrane capacitance, G_m is the membrane conductance, and $G_p(t)$ is the photoconductance that is a function of time. An exponential decay of the photoconductance results in the following equation:

$$I = C\frac{dV}{dt} = -VG_m - VG_b - VG_pe^{-t/\tau} + V_bG_b, \quad (1)$$

where V is the observed voltage at time t, G_p is the initial photoconductance and τ is its lifetime. The depolarization rise is the result of the additional separate parallel conductance after the light, G_p, which increases the total conductance. If this conductance did not decay, the membrane would depolarize exponentially to a final voltage determined by the new conductance ($G_m + G_p$). With a finite lifetime for G_p, the membrane is depolarized by the battery when G_p decays. The equation was solved numerically to fit the data. The solid line in Fig. 1 is the fit to the data using $G_p = 2 \times 10^{-9}$ mho and $\tau = 0.3$ s. The amount of charge displacement in the photocurrent, I_p, can be calculated by $q = \int_{\infty}^{0} I_p dt$. Assuming that the voltage change is small compared to the external applied voltage this integral gives $q = VG_p\tau$. Alternatively, q can be approximated by simply calculating the charge necessary to depolarize the membrane capacitance by the maximum observed photodepolarization. These two methods give similar values; the calculations using the results shown in Fig 1 give 0.9×10^8 and 0.6×10^8 ions, respectively. These values are about one third that of the number of ions required to form the interfacial photovoltage, 2.4×10^8 (calculated by $q = CV$ for a 7-mV photovoltage).

In this description, once the photoconductance has decayed, the membrane voltage recovers with the system RC time $= (1/G_m + 1/G_b + 1/G_v)C$. This was verified experimentally; the time constant for the decay of the observed photovoltage was found to be equal to the system time constant, when measured with the same membrane. The time at which the maximum depolarization occurs is dependent on the magnitude of the photoconductance, its lifetime, and the system time constant. This was observed experimentally by reducing the system time constant by decreasing the resistance of the voltage supply. The finite lifetime of the photoconductance could be the result of escape of the ions into the aqueous phase, protonation to form neutral species or simply stopping in the polar region. In any case, the finite system RC time and the current source will inevitably lead to repolarization of the membrane. Fits of the equation to the data resulted in variation of the photoconductance lifetime from 30 to 300 ms with different membranes.

Removal of Oxygen

When the oxygen concentration was decreased to $<10^{-6}$ M by the addition of glucose oxidase, catalase, and glucose, the slowly rising photodepolarization shown in Fig. 1 was eliminated and a small fast-rising photodepolarization was observed that also decayed with the system RC time constant. The magnitude of this photodepolarization vs. applied voltage is also shown in Fig. 2. The addition of the enzymes without glucose (and vice versa) had no significant effects. Measurements on a faster time scale have shown the risetime of the anaerobic signal to be <1 μs (data not shown).

Ferricyanide

Electron transfer from photoexcited MgOEP in a bilayer to aqueous ferricyanide results in interfacial ions that recombine within 2 ms (Hong, 1976). However, when ferrricyanide is added to both aqueous phases, photovoltages result at each interface that are equal but of opposite signs and thus cancel at the electrodes. That is, the system is vectorial: we can only measure electric field components perpendicular to the membrane. Thus, with ferricyanide in both aqueous phases, it is possible to observe photocurrents due to the movement of ions in the membrane without the interference of photovoltages from the interfacial ions. The addition of 5 mM ferricyanide to both aqueous phases, even in the presence of oxygen, eliminated the slow photodepolarization. Instead, a very small fast rising photodepolarization was observed that decayed with the system RC time constant. The amplitude of this photodepolarization was only 10% the slow photodepolarization (Fig. 2). Removal of oxygen when ferricyanide was present on either one or both sides had little effect on the photocurrent. Both of these results confirm that ferricyanide competes effectively with oxygen for electron transfer from the excited state (Ilani et al., 1985). This competition results in the elimination of that fraction of the photocurrent due to oxygen.

When 5 mM ferricyanide was added to only one aqueous phase, charge separation occurs within 100 ns at one interface and a photovoltage of 5–10 mV is observed which is independent of the membrane voltage (Hong and Maurzerall, 1976). Any membrane potential dependent photo-

voltage, i.e., photocurrent, can be easily measured only after the membrane potential independent photovoltage has decayed, ~2 ms. The photocurrent observed after 2 ms was equally small whether ferricyanide was present in either one or both aqueous phases.

Methyl Viologen

The decay of the interfacial photovoltage is much slower with MV^{+2} as the electron acceptor than with ferricyanide (Liu and Mauzerall, 1985). Thus the lifetime of P^+ is much longer with MV^{+2} as the acceptor. Fig. 3 shows that the addition of methyl viologen (MV^{+2}) in the presence of oxygen had the opposite effect to ferricyanide on the photocurrent. With MV^{+2} in either one or both aqueous phases, the photodepolarization amplitude increased.

When 8 mM MV^{+2} is added to only one aqueous phase, typically a photovoltage of 5 mV is produced within 1 μs, which decays with distributed kinetics (Liu and Mauzerall, 1985). When the membrane was polarized before the laser pulse, the observed photovoltage was dependent on the membrane polarization and appeared to be a summation of a membrane voltage independent photovoltage, or true photovoltage, and a membrane voltage dependent photovoltage, or photocurrent. As shown in Fig. 3, this photocurrent was ~2.5 times greater than that observed with no MV^{+2} and approximately 1/2 that observed with MV^{+2} on both sides.

The photocurrent increase, observed when MV^{+2} is added to either one or both aqueous phases, is not observed if oxygen is removed (Fig. 4). The anaerobic photocurrent at 50 ms is <20% of the aerobic photocurrent (Fig. 4 *A, C* vs. *D, F*) even though the decay of P^+ is incomplete (shown by the measurement with no membrane polarization, (Fig. 4 *B* and *E*). In addition, the anaerobic photocurrent with MV^{+2} was less than the aerobic photocurrent without MV^{+2}.

$MgOEP^+$ Trapping

Any P^+ that crosses the bilayer can be trapped by adding ferrocyanide to the aqueous phase opposite the electron acceptor since ferrocyanide rapidly reduces P^+ (Hong and Mauzerall, 1976). Upon adding ferrocyanide to the aqueous phase opposite to that containing MV^{+2}, as in Fig. 4, no change in the decay was observed (data not shown). The photovoltage decay remained independent of the membrane voltage.

The results obtained using ferricyanide as the electron acceptor, with ferrocyanide in the opposite aqueous phase, are more complex. Fig. 5 shows the results with ferricyanide in one aqueous phase and ferrocyanide in the other and with oxygen removed. Photovoltage is produced immediately after the flash due to the interfacial charge separation at the ferricyanide side and decays quickly on the time scale of the data shown. With ferrocyanide in the opposite aqueous phase, ~20–30% of the initial photovoltage decays

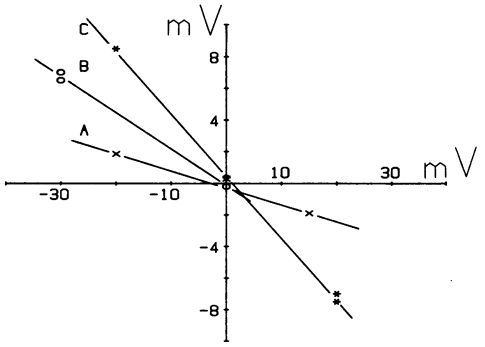


FIGURE 3 The maximum voltage change observed is plotted vs. the membrane voltage before the flash. (*A*) aqueous buffer on both sides of the membrane with no other addition (*B*) with 8 mM methyl viologen in one aqueous solution; (*C*) with 8 mM methyl viologen in both aqueous solutions. All the data were obtained with the same membrane.

with the system time constant. In addition, a membrane voltage dependent photovoltage, or photocurrent, is observed with decays with the system time constant (Fig. 5 *D, E, F*). These results are not necessarily due to the trapping of P^+ because when ferrocyanide is added without ferricyanide, a similar but smaller photovoltage and photocurrent are produced. The ferrocyanide photovoltage is typically 10–20% of the ferricyanide photovoltage, with the polarity expected for electron transfer from the ferrocyanide to the membrane and the magnitude decreases by about one half when oxygen is removed. Nevertheless, a very small (0.2–0.5 mV) photovoltage that rises within 5 ms is observed when ferricyanide and ferrocyanide are on

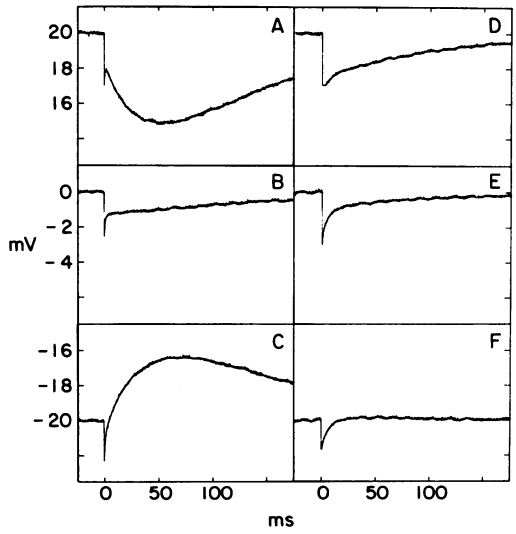


FIGURE 4 The membrane voltage is plotted vs. time as in Fig. 1, but with different membrane voltages before the flash indicated on the ordinate. (*A, B, C*) with 8 mM methyl viologen in one aqueous solution and different applied voltage as given on the ordinate; (*D, E, F*) with 8 mM methyl viologen in one aqueous solution and glucose oxidase, catalase, and glucose in both aqueous solutions and again with different applied voltages. All the data were obtained with the same membrane. The fast negative peak in *D* was offscale. Measurements on a faster time scale showed the fast neg peak to be identical in all six conditions.

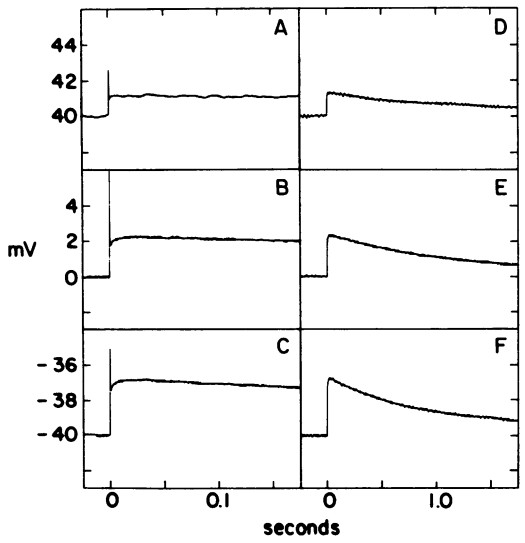


FIGURE 5 The membrane voltage is plotted vs. time as in Fig. 4. These data were obtained with 5 mM ferricyanide in one aqueous solution, 5 mM ferrocyanide in the other and glucose oxidase, catalase and glucose in both aqueous solutions. *A, B, C* time scale is ten times faster than *D, E, F*. The membrane voltage before the flash is given on the ordinate. On the time scale of the data shown, this was either a short positive spike or missing entirely. The spike shown in *A, B,* and *C* is real, but is not accurately shown due to the slow time scale. All the data were obtained with the same membrane.

opposite sides (Fig. 5 *A, B, C*). The addition of ferrocyanide to the ferricyanide eliminated all of these effects. The time necessary to reform the membrane if it broke, usually only one minute, was sufficient to contaminate the ferricyanide with ferrocyanide from the opposite side.

Proton Gradient

Measurements in the presence of a pH gradient across the membrane were undertaken to determine if P^+ moves from one interface to the other with the selective transport of either H^+ or OH^- ions (Young and Feldberg, 1979). If a bilayer component selectively transports ions across the bilayer, a membrane voltage will be observed, the value of which will approach the Nernstian limit as equilibrium is approached.

These measurements were performed by adding hydrochloric acid to one aqueous phase with equal amounts of an electron acceptor present in both aqueous phases. The pH was measured at the end of the experiment. The pH gradient did not produce any detectable voltage in the dark. The light pulse produced P^+ at both interfaces resulting in equal but opposite photovoltages at the two interfaces. Any resulting net photovoltage would be attributed to the pH gradient and thus to selective ion transport. This argument requires that the photovoltages at each interface cancel each other over the entire decay. Thus it is possible to observe an apparent photovoltage if the decay of the photovoltage at the one interface changes by as little as 5% on decreasing the pH. Calculated plots of equal photovoltages at opposite interfaces, but with the first order decay rate constant, k, differing by 1–10%, show a small broad maximum at about $t = 1/k$.

Fig. 6 shows the results obtained with MV^{+2} as the electron acceptor on both sides of the membrane and a pH gradient across the membrane in the presence of oxygen. Similar results were obtained when the oxygen concentration was decreased. A positive voltage would arise from H^+ transport or OH^- counter transport. Even in the presence of oxygen, where it could be suggested that O_2^- transports H^+ ions across the membrane, there is not more than 2 mV attributable to the pH gradient. Perfectly selective H^+ or OH^- transport would have resulted in a photovoltage of ~30 mV. Notice that this photovoltage rises at the same rate as the photodepolarization in Fig. 4. Similarly, it decays with the system time constant.

Measurements with ferricyanide as the electron acceptor also gave rise to small photovoltages, possibly attributable to the pH gradient. These measurements were made with various sequences of addition. One sequence was used which also determined the effect of pH on the photovoltage decay: ferricyanide was added to one aqueous solution, the pH of the opposite aqueous solution was then decreased by adding an aliquot of hydrochloric acid, ferricyanide was then added to the low pH side, and finally ferrocyanide was added to the high pH side. The photovoltage and decay was recorded after each addition. The first and last measurements in this sequence are of the photovoltage decay at the low and high pH, respectively. The decay

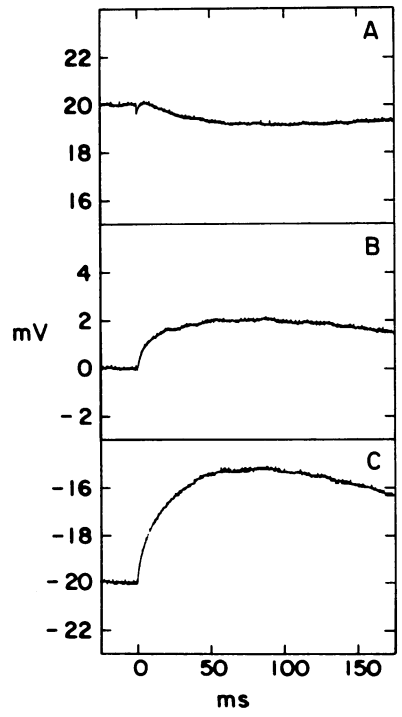


FIGURE 6 The membrane voltage is plotted vs. time as in Fig. 4. (*A, B, C*) with 8 mM MV^{+2} in both aqueous solutions and one aqueous solution at a lower pH so that selective transport would result in a positive voltage. The measured pH difference was 0.45. All the data were obtained with the same membrane.

of the interfacial photovoltage at pH 5.8 and 6.8 was the same within 2% (data not shown). Fig. 7 shows the results obtained after the addition of ferricyanide to both solutions and hydrochloric acid to one; thus the photovoltage would be due to the pH gradient. Again, this photovoltage is much smaller than the Nernstian voltage. However, the decay of this photovoltage was less than the system time constant, unlike the results with MV^{+2}. This faster decay is consistent with a faster protonation or deprotonation reaction.

DISCUSSION

The observed depolarization of the membrane after the flash is interpreted as a result of movement of photoproduced ions across the bilayer and/or the aqueous interface in response to the membrane polarization. Such a movement of ions in response to the membrane potential can be viewed as an increase of the membrane conductance or as a displacement current, either of which depolarizes the membrane. Thus, these measurements of membrane voltage dependent photovoltage are equivalent to the direct measurements of photocurrent with much longer light durations (1/60 s, Lutz et al., 1974). Conductivity measurements in acetonitrile have shown that quenching of the triplet state of MgOEP by O_2 produces ions, assumed to be P^+ and O_2^-, in low yield (Mauzerall, 1976). Since oxygen is soluble in the bilayer, these ions could be formed within the bilayer and the movement of either one or both ions in response to the applied membrane potential gives rise to the photocurrent. Once the ions reach the aqueous phase their movements no longer effect the membrane current.

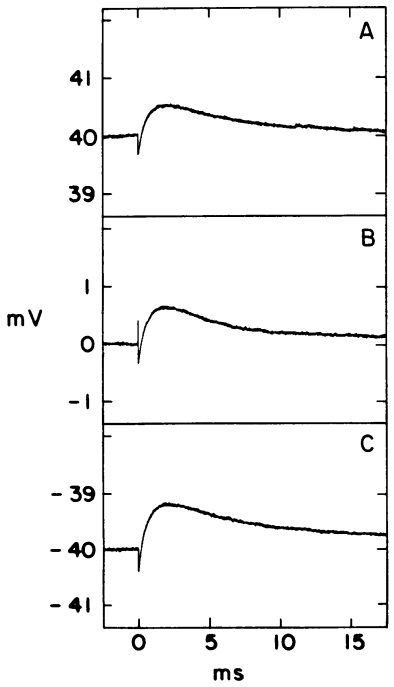


FIGURE 7 The membrane voltage is plotted vs. time. These data were obtained with 5 mM ferricyanide in both aqueous solutions. A 1.0-M solution of HCl was added to one of the aqueous solutions so that selective transport would result in a positive voltage. The measured pH difference was 1.03. All the data were obtained with the same membrane.

To determine whether the movement of P^+ or O_2^- ions is responsible for the photocurrent, its dependence on oxygen and other electron acceptors was examined. Experiments were also undertaken to observe any P^+ ion movement to the opposite interface by trapping it there with ferrocyanide, which is known to react rapidly with P^+. Voltages possibly due to the selective cotransport of H^+ or OH^- ions with a pH gradient across the bilayer were also investigated.

Removal of Oxygen/Addition of Electron Acceptors

When oxygen is removed, or the electron acceptor ferricyanide added, the photodepolarization decreased and what little remained had a fast rise time, <1 μs. This residual photocurrent could be attributed to P^+P^- ions, formed following excitation of P, which can separate in <1 μs by charge transfer within loose aggregates of the MgOEP. By loose aggregates we mean lipid intercalated porphyrins along the interface with a small distribution of depths. When oxygen is present it scavenges P^- and the O_2^- and P^+ ions separate more slowly by ionic motion. This results in the observed slow depolarization. When ferricyanide is also present, it effectively competes with oxygen as the electron acceptor and eliminates the slow photodepolarization attributed to oxygen. Ferricyanide competes with the interfacial oxygen, but probably not with oxygen deeper in the bilayer, because of the limited distance over which electron tunneling occurs (Mauzerall, 1976). Thus, any MgOEP that is deeper in the bilayer could produce O_2^- and P^+. Therefore, the large decrease in the photocurrent with addition of ferricyanide indicates that MgOEP is almost entirely in the interfacial polar region. A similar conclusion was reached in a study of Chl b containing vesicles by using low temperature site-selection fluorescence spectra and observing specific solvent dependent sharp lines (Funfschilling and Walz, 1983). Energy calculations also place the Chl in the region of the glycerol ester groups (Brasseur et al., 1984).

The large decreases in the photodepolarization upon ferricyanide addition strongly suggests that only O_2^- ion movements are responsible for the slow photocurrent; when ferricyanide is present P^+ is still produced but O_2^- is not. It could be argued that the photocurrent is decreased because of the decreased lifetime of P^+ (<20 ms) in the presence of the ferricyanide (due to traces of ferrocyanide in the ferricyanide) but this explanation is unlikely. It would not explain the small, fast rising photocurrent observed upon symmetrical ferricyanide addition, whereas movements of P^+ within an aggregate would.

When MV^{+2} is the electron acceptor instead of ferricyanide, and oxygen is present, the lifetime of P^+ is much longer. The longer lifetime has been attributed to the

encounter-limited reaction of MV^+ with O_2 producing O_2^- which is less reactive with P^+ (Michaelis and Hill, 1933; Liu and Mauzerall, 1985). The presence of MV^{+2} as an electron acceptor results in the same amount of P^+ in the membrane, as compared with ferricyanide (Ilani and Mauzerall, 1981), but increases the photocurrent. The reduced MV^+ produced by the flash reacts with oxygen to produce O_2^-. Consequently, the yield of O_2^- is much greater than from the direct reaction of oxygen with the excited porphyrin. The additional O_2^- results in additional photocurrent. But for the O_2^- to give rise to photocurrent, it must be generated within either the hydrocarbon region or the interfacial region. Thus the MV^+ and, by inference, the MV^{+2} must also be there. The presence of MV^+ in the interfacial region is supported by the report of the photoinduced transport of MV^{+2} across dihexadecyl phosphate vesicles (Lee et al., 1983). The movement of MV^+ could also be responsible for a photocurrent. However, any oxygen present will quickly react with the MV^+. Finally, since the photocurrent is largely eliminated with the removal of oxygen, even though P^+ and MV^+ are still formed and live at least 0.1 s, the photocurrent is largely attributed to O_2^-.

Our claim, that O_2^- is the oxygen ion responsible for the photocurrent, is supported by reports of O_2^- permeability across lipid vesicles (Rumyantseva et al., 1979; Takahashi and Asada, 1983). In addition, the lifetime of O_2^- in the bilayer should also be sufficient for this role. The observed second order rate constant for the disproportionation of superoxide free radicals in aqueous solutions at pH 7 is 10^5 M^{-1} s^{-1} (Bielski, 1978). For a concentration of O_2^- in the membrane of about 10^{-5} M, the lifetime would be ~1 s, assuming the rate constant is the same. The rate constant for disproportionation should be even lower in the nonprotic, lower dielectric of the membrane.

$MgOEP^+$ Trapping

It is known that ferrocyanide rapidly reduces P^+ at the bilayer-water interface (Hong and Mauzerall, 1976). Thus, if ferrocyanide is added to one aqueous phase and an electron acceptor to the other, P^+ movement or interpigment charge transfer across the bilayer can be detected as trapped charge separation across the membrane. The photovoltage from charge separation across the entire bilayer should be larger, perhaps 10 times or more, than the photovoltage from charge separation across a single interface. Unfortunately, quantitative estimation of the increase of the voltage due to separation across the entire bilayer is difficult because of the uncertainty of both the exact location of the P^+ in the interfacial region and the inhomogeneity of the dielectric constant across the bilayer (Raudino and Mauzerall, 1986). Nevertheless, if the aqueous oxidized species on one side and the aqueous reduced species on the other side cannot cross the membrane the voltage due to this charge separation will decay with the system time constant even though the species produced still exist. If the crossing of P^+ is fast relative to its lifetime, the P^+ will equilibrate at both interfaces and that at the ferrocyanide interface will be reduced rapidly. Thus, a large photovoltage, >50 mV, should rise with the crossing time and decay with the system time constant. If the crossing of the P^+ is slow, then there should be no effect of the trapping agent on the photovoltage decay.

When MV^{+2} was present in one aqueous phase, addition of ferrocyanide to the other aqueous phase had no effect on the photovoltage decay. Thus no trapping is observed, even though P^+ lives at the MV^{+2} interface for more than 0.1 s.

However, some ambiguity exists in the results obtained when ferricyanide is the electron acceptor instead of MV^{+2} but this does not alter our conclusion. The photovoltage and photocurrent observed with ferrocyanide opposite the ferricyanide are about twice as large as when only ferrocyanide is present. The fast photovoltage is probably due to the reduction of P^+, generated at the same interface, by the ferrocyanide and the photocurrent to small movements of the remaining P^- within the aggregate. Whereas, the very small and slow photovoltage observed (Fig. 5 *A, B, C*) is unique to the opposition of ferrocyanide and ferricyanide. These results could be explained by trapping at the ferrocyanide interface of <5% of the P^+ generated at the ferricyanide interface. Nevertheless, it is not clear why trapping should be observed with ferricyanide but not with MV^{+2} as the electron acceptor, especially since the lifetime of P^+ is shorter with ferricyanide.

Proton Gradient

When a pH gradient exists across the bilayer, any species that selectively transports H^+ or OH^- ions across the bilayer will produce a membrane voltage that cannot exceed the Nernstian limit of the pH gradient but may be less due to imperfect selectivity. If the transporting species is short-lived and no other processes reverse the ion separation, any voltage thereby produced will decay with the system time constant. The voltage may decay in less time as the result of any process that reverses the charge separation.

The pH gradient across phosphocholine bilayers containing MgOEP did not produce any detectable voltages in the dark. Thus, the neutral MgOEP species did not selectively transport H^+ or OH^- ions. When an electron acceptor was present on either one or both sides, reducing the pH of one aqueous phase resulted in <10% of the photovoltage possible by selective transport of H^+ or OH^-. This voltage was consistently observed with the polarity expected for selective transport. The voltage observed with ferricyanide had a rise time of ~1 ms, which is ~10 times faster than the rise of the photodepolarization. Likewise, the decay was ~10 ms, which is much faster than the system time constant. However, the pH-dependent voltage observed with MV^{+2} as acceptor had a rise and decay time similar to that of the photodepolarization. Thus, it is likely that O_2^- is the carrier here. We note that it is not necessary

for the proton charge to completely cross the membrane to result in Nernstian potentials. It is only necessary for the proton or hydroxyl charge to cross a bilayer-water interface to observe at least part of this potential. The traditional glass electrode works in this manner. Thus the fast time constants observed with ferricyanide and the slow time constants observed with MV^{+2} for the voltage to rise and decay, may represent different interfacial proton transfer reactions such as protonation of O_2^- vs. OH^- transfer to $MgOEP^+$.

The slow time scale studied in these experiments represents the limit of the voltage method due to the limiting RC time constant of the membrane (~1 s). Consequently, these results set the lower limit for the diffusion of $MgOEP^+$ across lecithin bilayers, or of the charge transfer by electron exchange with other MgOEP molecules, in the range of 0.1–0.5 s. It has been reported that the diffusion time for the neutral complex of ferric protoporphyrin IX dimethylester with an anion such as hydroxide across lecithin vesicles is 0.3 s (Runquist and Loach, 1981).

Chl *a* also reacts with oxygen but no photocurrents were observed when Chl *a* was substituted for MgOEP in the membrane. This may be due to a transfer of H^+ from the C_{10} of Chl *a* to O_2^-, which results in neutral products (Quinlan, 1971). In fact, similar photocurrents recently reported with Chl *a* containing bilayers were observed only in the presence of NaI, and were not attributed to Chl^+ but to polyiodide ions (Arrietas et al., 1985).

In contrast with the results of this work, faster charge transfer across lipid vesicles mediated by photoexcited Chl *a* has been reported with a rate of $10^4\ s^{-1}$ (Ford and Tollin, 1983). The mechanism for this charge transfer was postulated as electron exchange between Chl *a* and Chl $a^{+\cdot}$ within the membrane. This interpretation is, however, based on complicated absorbance changes which measure nonselectively various Chl ions, neutral species, and triplets at both interfaces and in the hydrocarbon region of the bilayer. Moreover, the small unilamellar vesicles (SUV) may provide a very different interfacial environment due to the high curvature. If so, SUV's are a poor model for cellular or organelle membranes.

Measurements of charge movement across planar bilayers of glycerol monooleate (GMO) containing MgOEP were interpreted as resulting from P^+ and P-OH crossing the bilayer within 1 ms (Young and Feldberg, 1979). The $MgOEP^+$ species was proposed to act as a carrier for H^+ or OH^- and facilitated many ion translocations during its lifetime, 0.5–1 s. The discrepancy between this work and that of Young and Feldberg may be due to significant differences in the lipids used; our results are obtained with phosphotidylcholine rather than glycerol monooleate. The GMO is much more fluid and the membranes made with it have a much shorter lifetime, on the average about that of a single measurement. It is also unlikely that their method of oxygen removal is as thorough as the enzymatic method.

Thus we conclude that the relative mobility of ions across our bilayer system is $O_2^- > MV^+ > MgOEP^+$. We also conclude that the observed photocurrents are largely due to superoxide anion, and perhaps other impurities. Finally, we can set the lower limit for the transmembrane transit time of MgOEP in the range of 0.1–0.5 s.

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