THE POTENTIAL SPAN OF PHOTOREDOX REACTIONS OF PORPHYRINS AND CHLOROPHYLL AT THE LIPID BILAYER-WATER INTERFACE

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ABSTRACT Lipid bilayers containing chlorophyll (Chl) or magnesium octaethylporphyrin (MgOEP) and separating solutions containing varying amounts of differing acceptors are illuminated by a dye laser pulse (FWHM 0.3 μs) at 590 mm. Interfacial charge transfer is measured at the first current peak in a voltage clamp circuit. The constants describing the hyperbolic saturations of the charge transferred by differing acceptors are only weakly related to the redox potential of the acceptors. An assymetric molecule, anthraquinone-2-sulfonate, is over 20 times as effective in accepting the electron as is the symmetrical anthraquinone–2,6 -disulfonate. In contrast to this variable effectiveness, the maximum amount of charge transferred as a function of acceptor redox potential is constant up to a cut-off value: -0.6 V (vs. standard hydrogen electrode) for MgOEP and -0.5 V for Chl. The reversible redox potential of MgOEP in the bilayer was determined by following both the decrease in photoactivity and the transmembrane potential as a function of aqueous redox potential. It is $+0.77$ V for MgOEP and ~ 0.7 V for Chl (limited by stability). Thus, a total of 1.4 V of reversible redox potential (free energy) is obtained from 1.8 eV (internal energy) of the triplet excited state of MgOEP.

INTRODUCTION

Pulse irradiation of lipid bilayers containing prophyrins has been used to study interfacial electron transfer from the excited membraneous chromophores to ferricyanide present in the aqueous solution (Hong and Mauzerall, 1972, 1976; Hong, 1976; Mauzerall, 1979). The aim of the present study is to extend the above system to various electron acceptors in order to unravel the basic rules that govern the interfacial photochemical electron transfer processes. The chromophores used were magnesium octaethylporphyrin (MgOEP) and chlorophyll *a*. The excited pigment was shown to react with a very wide range of acceptors whose redox potentials are more positive than -0.7 V. The reversible redox potential of the pigment in the bilayer was determined to be $\sim +0.7$ V. Thus, a very large fraction of the excited state energy is converted to a chemical free energy at the interface. These results support a simple mechanism of interfacial charge transfer via electron tunneling.

MATERIALS AND METHODS

Membrane Cell

The membrane cell consisted of two halves of lucite chambers separated by a teflon sheet in which a hole of ~ 1.6 mm Diam was punched. The membrane was formed on the hole by the usual Rudin-Mueller

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brush technique (Mueller et al., 1963). The front walls of the cell were made of cover slips. The aqueous solutions on both sides of the membranes were mixed by magnetic stirrers.

Illumination

A rhodamine 6G flash lamp pumped dye laser ($\lambda \simeq 590$ nm, 0.3 μs full width half maximum) provided illumination. The beam was focused on the center of the hole in the teflon partition to avoid irradiation of the thick annulus of the membrane. Pulse intensity varied by 20% from shot to shot and could be varied several-fold intentionally by changing the high-voltage input of the laser pump. Monitoring of pulse intensity was affected by placing a scattering plane behind the cell. The scattered light was sampled by a calibrated photodetector (Lite-Mike Model 560B, EG&G, Inc., Salem, Mass.). The integrated output of the detector was recorded on a storage scope and its magnitude recorded manually on the simultaneous record of the electrical signal.

Electric Circuit

The electric circuit consisted of two calomel electrodes placed on the two sides of the membrane and connected to an operational amplifier (Zeltex 133). The 10^6 Ω resistor in the feedback loop was chosen so that the current through the amplifier produced a voltage adequate for the most sensitive scale of the wave-form recorder. The resulting time constant, ~10 μs, was adequate for this work. The output of the operational amplifier was connected to a fast wave-form recorder (Biomation Model 805), which was triggered by the laser activation mechanism. The digitized sweep was displayed on a scope and then transferred to a chart recorder for analysis.

Typical responses are shown in Fig. 1. A rigoruous analysis of the electrical signal was given in an earlier publication (Hong and Mauzerall, 1976). For the benefit of the general reader, some points of the analysis are reiterated here. After short-pulse light irradiation, electron transfer occurs at the interface with the solution that contains appropriate electron acceptors. This leads to an instantaneous (<100 ns) charging of that interface. Owing to the "voltage clamping" by the operational amplifier, current flows through the system to return the potential difference accross the membrane to 0 V. The polarized

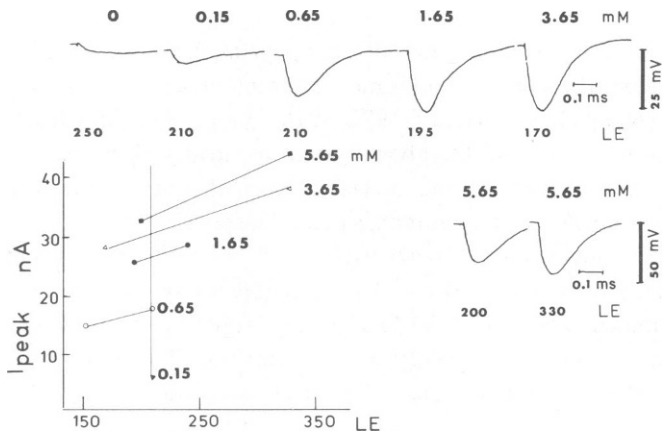

FIGURE 1 Photoresponse following flash illumination of a membrane containing MgOEP. The record shows the output of the operational amplifier at feedback resistance of 1 MΩ and feedback capacitance of 10 pF. The ferricyanide concentration on one side of the membrane was raised successively, as indicated above each record. The relative light energy (LE) for each flash is indicated below each record. Each unit corresponds to about 1 μJ. For the sake of brevity only one record for each ferricyanide concentration is shown, except for the 5.65-mM records. The aqueous solution contained 0.1 M NaCl and 1 mM phosphate pH 6.5. Inset: peak photocurrent vs. flash light energy. The peak currents at the indicated light energy were used for the analysis represented in Fig. 2.

membrane then relaxes by reverse charge transfer at the interface and/or crossing of the bilayer by the charged pigment. This results in a characteristic biphasic wave form (Mauzerall, 1979). On a time scale of <1 ms and in the absence of electron donors, the current as a function of time appears as a wave whose amplitude and form are determined by the amount of charge transferred at the interface, the access impedance (primarily the electrode impedance), and the membrane capacitance. Only if the reverse charge transfer is very rapid, i.e., occurring in $<\sim 100$ μs, does this process affect the shape of the curve. This is only seen when electron donors are added to the acceptor solution. The total shape of the signal is also filtered by the response time of the operational amplifier, which in our case was 10 μs, determined by the feedback resistance and capacitance. In principle, any constant point on the current wave record could be used as a measure of the amount of charge transferred after the pulse light irradiation, provided that the access resistance, membrane capacitance, and operation amplifier parameters remained constant. In practice, the peak current I_{peak} was used in this study as a parameter proportional to the extent of excited electron transfer through the interface.

Solutions

The membrane-forming solution consisted of 3% egg lecithin, 0.8% cholesterol (Sylvania Chemical Co., Millburn, N.J.) dissolved in a 10:1 decane-amyl ether mixture. In addition the solution contained ~5 mM MgOEP or ~2 mM chlorophyll *a*. The chlorophyll *a* was prepared as described by Masters and Mauzerall (1978) and checked for purity on a Cary 15 spectrophotometer (Cary Instruments, Fairfield, N.J.).

The aqueous solutions were prepared with doubly glass-distilled water and analytical grade solutes. The basic solutions contained 100 mM NaCl and 1 mM phosphate buffer adjusted to the required pH. In some cases, where metal ammonia complexes were used, 5 mM of NH_4Cl was added to the 100 mM-NaCl solution.

Electron Acceptor Chemicals

The following electron acceptor chemicals were used: potassium ferricyanide (Mallinckrodt Inc., St. Louis, Mo.), potassium cobaltocyanide, *N*-methyl nicotinamide (NMNA), hydroxy ethyl nicotinamide (HENA), nicotinamide adenine dinucleotide phosphate (NADP, Sigma Chemical Co., St. Louis, Mo.), methyl viologen (known as paraquat, recrystallized, Sigma), anthraquinone-1,5-disulfonate and 2,6-disulfonate disodium salts (1,5-AQDS and 2,6AQDS, recrystallized, (Eastman Organic Chemicals Div., Eastman Kodak, Rochester, N.Y.), 9,10 phenanthraquinone-3-sulfonate potassium salt (PQS, prepared by Michaelis and Schubert [1937]), anthraquinone-2-sulfonate (2-AQS, prepared by Michaelis and Schubert [1937]), napthoquinone-2-sulfonate potassium salt (NQS, Eastman), potassium chloroiridate, hexaamino cobalt chloride and hexamino ruthenium chloride (Alfa Div., Ventron Corp., Danvers, Mass.). The compounds without sources were prepared and purified by standard methods. The following three viologens were a gift from Dr. Stuart M. Ridley of ICI Ltd, Brackness, England: diquat, triquat and tetraquat. These viologens are α,α' dipyrridyls that are quarternized and thus cyclized by the following groups: diquat, 1,2-ethane; triquat, 1,3-propane; and tetraquat, 1,4-butane. Each acceptor was freshly prepared in an aqueous solution the concentration of which was at least 50 times higher than its final concentration in the solution at the membrane. The acceptors were added to one side of the membrane after it had thinned.

Redox Potential

The one-electron potential of the above electron acceptors is quoted from the literature whenever possible (Clark, 1960; Millazzo and Caroli, 1978). For the quinones the preferred reference is the one-electron redox potential, E_1 in Michaelis' nomenclature (Michaelis and Schubert, 1938). In some cases the polarographic data are quoted. The difference between the one- and two-electron potential is not large ($\leq$100 mV) and will not affect the conclusions of this paper. All values quoted are referred to the standard hydrogen electrode. In some experiments the redox potential of the solution facing the membrane was measured directly by means of a combination platinum-calomel electrode (Radiometer Co., Copenhagen, Denmark). The electrodes were calibrated by immersing them in a 0.1 m NaCl

solution also containing 2 mM of both potassium ferricyanide and ferrocyanide (E_0 = 0.40 V at 0.2 M ionic strength) (Hanania et al., 1967).

RESULTS

Relationship Between Photoresponse and Electron Acceptor Concentration

Fig. 1 shows a sample of raw data. The photocurrent after pulse irradiation of MgOEP-membrane is shown for consecutive increases in ferricyanide concentration on one side of the membrane. The peak photocurrent was used as a measure of the charge transferred (see Methods). The simultaneous measure of the relative flash light energy is indicated. For each ferricyanide concentration at least two photocurrent responses were recorded. The two or more responses were related to different pulse intensities by a linear approximation to determine the extent of the response at an arbitarily chosen intermediate intensity (see lower part of Fig. 1). The function of response vs. light energy is actually the complement of an exponential, but the difference between the true curve and the linear interpolation is small.

Fig. 2 represents the analysis of the data shown in Fig. 1. It is clear that saturation-type kinetics are observed, i.e., the current that can be elicited by a pulse irradiation of a particular energy is dependent upon electron acceptor concentration but it can reach only a limited

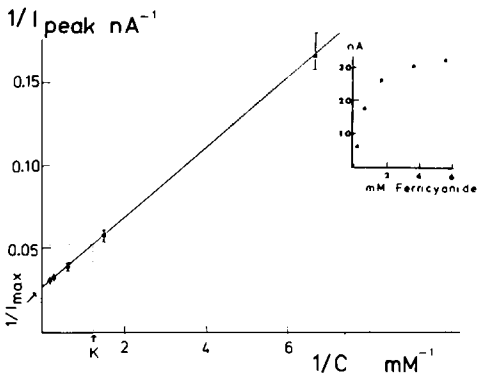

FIGURE 2

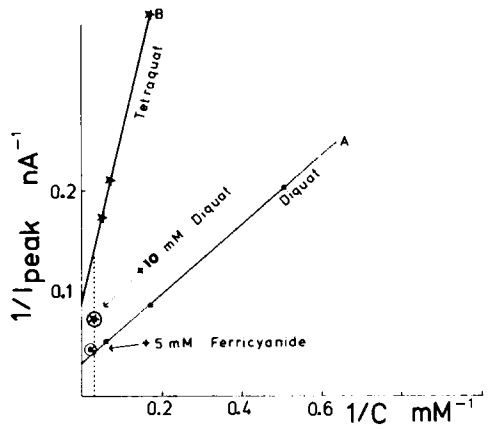

FIGURE 3

FIGURE 2 Double reciprocal plot of peak photocurrent vs. ferricyanide concentration. Inset: peak photocurrent response as function of ferricyanide concentration.

FIGURE 3 Double reciprocal plot of peak current vs. concentration for diquat (*A*) and tetraquat (*B*). At the end of each study 5 mM of ferricyanide or 10 mM of diquat, respectively, were added. The reciprocal of the photocurrent after each addition (encircled symbol) is shown in the Figure at a corresponding "weighted" reciprocal concentration as explained henceforth: since $1/K$ for ferricyanide is about 12 times lower than for diquat (Table I), the 5 mM ferricyanide was considered equivalent to ~60 mM diquat. Since this was added to 18 mM diquat solution the total equivalent diquat concentration was ~78 mM. Therefore the reciprocal photocurrent after ferricyanide addition is depicted at 1/78 mM^{-1}. Similarly, since $1/K$ for diquat is 2.5 times lower than that for tetraquat (Table I), the 10 mM diquat added was considered equivalent to 25 mM increment in tetraquat concentration. Added to a 22 mM tetraquat solution, the reciprocal photocurrent after the diquat addition is plotted at a point corresponding to 1/47 mM^{-1}. It can be seen that the photocurrent after diquat addition is about twice as high (and thus one-half the amplitude in the reciprocal plot) as the corresponding point on the tetraquat curve (dashed line).

value, I_{max}. As in the analogous Lineweaver-Burk analysis (Cleland, 1970), a plot of the reciprocal of the photocurrent response vs. the reciprocal of the electron acceptor concentration yields a straight line (Fig. 2) from which two parameters that characterize the acceptor with respect to the particular membrane can be obtained: I_{max} and K. $1/K$, the analog of K_M in the enzymatic-kinetics notation or of K_Q in the Stern-Volmer notation (Turro, 1965), is the concentration at which half of the maximal photocurrent is obtained. The saturation of linear, first-order systems, whether they are at equilibrium or in a steady state, is hyperbolic.

I_{max} and K for Various Electron Acceptors

Using various electron acceptors, we found that I_{max} was roughly the same for most of them. Since the absolute current observed may vary from experiment to experiment because of the many parameters to be controlled, it was desirable to have a direct comparison of the various acceptors. The method for quantitative estimate of I_{max} for a particular acceptor with respect to another is illustrated in Fig. 3. *A* represents the result of an experimental study of the I_{peak} vs. concentration for diquat. The encircled point near the ordinate represents the reciprocal of the photoresponse after addition of 5 mM ferricyanide to a solution containing 18 mM diquat (see legend, Fig. 3). From this experiment it could be inferred that I_{max} for ferricyanide is not greater than that for diquat. The inverse experiment (not shown in Fig. 3) of measuring the effect of addition of diquat to a solution containing ferricyanide proved the converse. It was thus possible to conclude that I_{max} for diquat and ferricyanide are indeed the same. In experiments with other electron acceptors, ferricyanide and diquat were used as reference materials to establish the value of I_{max}. Fig. 3 *B* demonstrates a case where I_{max} for another acceptor, tetraquat, is only half the value for diquat.

Table I summarizes the results of many experiments of the type shown in Fig. 3 carried out with various electron acceptors for membranes containing either MgOEP or chlorophyll *a*. The noteworthy features of this table are as follows: (*a*) I_{max} for the MgOEP membranes is the same for all acceptors that have redox potential of between +0.4 and −0.55 V. For an acceptor of redox potential of −0.6 V (tetraquat), I_{max} drops by 50%; and for an acceptor of redox potential of −0.8 V [e.g., $Co(CN)_6^{-3}$] (or even more negative), I_{max} is zero. (*b*) In chlorophyll *a* membranes, the drop in the photocurrent occurs at a more positive redox potential, as is evident from the fact that tetraquat and triquat do not elicit any photocurrent in those membranes. (*c*) There are exceptions to the above rules. A striking exception is the $Ru(NH_3)_6^{+3}$ complex, which has a one-electron redox potential of +0.06 V and yet elicits an I_{max} of only 30% in the chlorophyll membranes. Second, AQS in the chlorophyll membranes produces I_{max} of only 70%, even though its electron redox potential E_1 at pH 7 is ~−0.2 V. A possible explanation for these exceptions is discussed later. (*d*) The K for the various acceptors studied vary by three orders of magnitude. In spite of this wide range, the K for the various acceptors is generally the same within a factor of 1.5 for both the MgOEP and the chlorophyll membranes. Two exceptions to this rule are paraquat (where K is 0.035 mM^{-1} for chlorophyll membranes, compared with 0.1 mM^{-1} for MgOEP membrane) and $Ru(NH_3)_6^{+3}$ (where K is 0.2 vs. 0.9 mM^{-1}, respectively) (see Table I). If one treats anthraquinone sulfonate as an unusual exception, the variation in the K covers two orders of magnitude. Furthmore, if the acceptors are further classified as cations and anions, the variation within each group is limited to approximately one order of magnitude with the anions having the higher K.

TABLE I

I_{MAX} AND K FOR VARIOUS ACCEPTORS TO MgOEP AND CHLOROPHYLL *a* CONTAINING MEMBRANES

Acceptor*	E_0 vs. SHE	Reference	MgOEP		Chlorophyll *a*	
			I_{max}	K	I_{max}	K
	V		*(%)*	*(mM^{-1})*	*(%)*	*(mM^{-1})*
HENA	−0.8‡		0	—		
NMNA§	−0.84	*A*	0	—		
NADP	≃0.80‖	*B*	0	—		
$Co(CN)_6^{-3}$	−0.80	*C*	0	0		
Tetraquat§	−0.64	*D*	50	0.04	0	—
Triquat§	−0.55	*D*	100 100	0.06, 0.05	0	—
Paraquat§	−0.44	*E*	100, 90	0.1, 0.1	100	0.035
Diquat§	−0.34	*D*	100 100	0.12, 0.13, 0.125	100	0.1
1,5-AQDS¶	−0.37	*F*	100	0.13, 0.15		
2-AQS**	−0.26	*G*	90, 100	35, 31, 26	70, 75	26, 25
2,6-AQDS¶	−0.20	*F*	100	1.5		
3-PQS**	−0.05	*H*	100	4.0	100	5.5
2-NQS**	0.05‡		100	1.5	100	2.5
$Ru(NH_3)^{+3}$	0.07	*I*	100	0.9	30	~0.2
$Co(NH_3)_6^{+3}$	0.10	*J*	100	0.14		
$Fe(CN)_{6-}^{-3}$	0.40	*K*	100‡‡	1.6, 1.35, 1.5	100‡‡	1.2

Each number under MgOEP and Chlorophyll-*a* represents the result of one complete I_{max} vs. concentration study, as explained in the text. Some membranes broke before the addition of test acceptor for determination of I_{max}. There are therefore more K than I_{max} figures.

*For meaning of abbreviations see Methods.

‡Estimated.

§Polarography in aqueous solutions (one electron, reversible).

‖Measured E_0 of NAD.

¶Polarography in aqueous solutions (two electrons, reversible).

**E_1 values at pH 7.0.

‡‡By definition.

References: *A*, Burnett and Underwood, 1965*a*; *B*, Burnett, and Underwood, 1965*b*; *C*, Handbook of Physics & Chemistry, 36th Edition, 1954; *D*, Ridley, personal communication, 1979 (recent work by R. C. Prince [to be published] shows the polarographic waves to be more complex than single electron reductions); *E*, Clark, 1960; *F*, Standard Methods of Clinical Chemistry, 1965; *G*, Gill and Stonehill, 1952; *H*, Michaelis and Schubert, 1937; *I*, Meyer and Taube, 1968; *J*, Milazzo and Caroli, 1978; *K*, O'Reilly, 1973.

Determination of the Porphyrin Redox Potential in the Membrane

Two experimental approaches were used to estimate the redox potential of the porphyrin in the membrane, V_0. One method was based upon the notion that the magnitude of the photoresponse is dependent upon the porphyrin concentration in the membrane. Thus, oxidizing the pigment to the cation in the dark will result in a decrease of the photoresponse. It was therefore expected that if the solution redox potential, V_r, is brought to the level of the porphyrin redox potential, V_0, the photoresponse would be cut by half. Thus, from the data shown in Fig. 4 it can be inferred that the redox potential of MgOEP in the thin lipid membrane is +0.76 V.

The second method was based upon measurement of membrane potential arising from the generation of P^+ ions at one interface. The concentration of P^+ at the other interface was kept

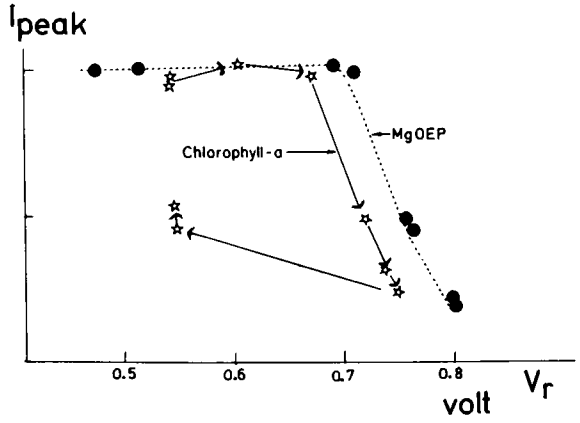

FIGURE 4 Photoresponse vs. redox potential of the solution containing either 5 mM ferricyanide (filled circles, MgOEP membranes) or 10 mM diquat (open stars, chlorophyll *a* membranes). The redox potential of the solution, V_r, was varied by adding chloroirridate and ferrocyanide (MgOEP), or chloroirridate and ascorbate (chlorophyll *a*). In addition to 100 mM NaCl the solution contained 1 mM phosphate (MgOEP), pH 6.5 or 5 mM acetate (chlorophyll *a*), pH 5.0. The arrows on the chlorophyll *a* curve indicate chronological sequence of the experimental procedure.

at zero by introducing ferrocyanide into the solution facing that interface. With the aid of the so called "constant field equation" (Goldman, 1943; Hodgkin and Katz, 1949), it was possible (see Appendix) to derive the following relationship between membrane potential, V_m, and redox potential of the solution, V_r:

$$V_m = (RT/F) \ln \{[Me^y/(1 + e^y)] + 1\},$$

where

$$y = (V_r - V_0)F/RT,$$

and

$$M = U_P \cdot C_P/\Sigma U_i C_i. \quad (1)$$

V_0 is the redox potential of porphyrin. U_P and C_P are the mobility and the concentration respectively of the porphyrin in the membrane. $\Sigma U_i C_i$ refers to mobilities and concentrations of all ions present in the membrane other than P^+. $\Sigma U_i C_i$ determines the membrane conductance in the absence of P^+. The curves in Fig. 5 represent solutions of Eq. 1 for $V_0 = 0.8$ and various M as indicated on the curves. It can be seen that the experimental data could be fit to Eq. 1 if V_0 was 0.8 V and M somewhere between 100 and 50. The sensitivity of this method enabled the determination of V_0 only within the limits of ±0.05 V.

The usefulness of the above mentioned methods was limited by the fact that some irreversible chemical changes took place if the pigments were exposed for a long time to oxidizing solutions. This was particularly evident at higher pH. For instance, at pH 7.5 the deterioration of the MgOEP occurs rapidly as was evident by the fact that the membrane potential developed in response to addition of chloroirridate to one side of the membrane fell within a few minutes. Reducing the pH to 6.5, however, was enough to ensure a stable reading

of membrane potential that was reversible to back-and-forth reduction-oxidation steps. At pH 6.5 the porphyrin deteriorated only if it was kept in the oxidizing environment for a few hours, as was evident by the decrease of membrane potential and by the fall of the photoresponse on re-reducing the aqueous solution.

In the case of the chlorophyll *a* membranes the situation was worse because reversibility was poor even at lower pH. Thus, at around $V_r = 0.7$ V the photoresponse declines by ~50% but re-reduction even within minutes did not fully restore the photoresponse (see Fig. 4). The membrane potentials generated were small (probably owing to the low mobility of chlorophyll in the membrane, i.e., small M in Eq. 1) and therefore not very useful in determining the redox potential.

In conclusion, the MgOEP in the thin lipid membranes has a redox potential of 0.8 ± 0.05 V. This is to be compared with a value of 0.78 (0.54 vs. Standard Calomel Electrode) determined polarographically (Davis, 1978) in butyronitrile of dielectric constant 20, and with a value of 0.66 V determined potentiometrically (Fuhrhop and Mauzerall, 1969) in methanol solutions of dielectric constant 33. Since the values quoted from the literature contain an unknown junction potential of up to 0.1 V (Felton, 1978), it is reasonable to conclude that in the membrane interface the redox potential of the MgOEP is about the same as in a polar solvent. This finding tends to support the notion that the porphyrin-acceptor electron transfer occurs at the interfacial region of the thin lipid membrane (dielectric constant 20–30) rather than deeper in its interior. From Fig. 4 the redox potential of chlorophyll *a* in the lipid membrane appears to be ~0.7 V with an uncertainty of ~±0.1 V. The polarographically determined oxidation potential for chlorophyll *a* in various solvents varies between 0.75 and 0.86 V (Felton, 1978).

DISCUSSION

The data show that the total charge transferred across the membrane interface during a short pulse of light is a hyperbolic function of the concentration of acceptor in solution. The saturation constant obtained from the data is a weak function of the redox potential of the acceptor, and the maximum charge transferred is independent of redox potential until a limit is reached. We shall discuss these results in turn. The following mechanism fits the data:

$$\mathrm{P}|\underset{k_{2r}}{\overset{\theta}{\rightleftharpoons}}\mathrm{P}^*|, \tag{2}$$

$$\mathrm{P}^*|+|\mathrm{A}\xrightarrow{k_3}\mathrm{P}^+|\,\mathrm{A}^-, \tag{3}$$

$$\mathrm{P}^+|\,\mathrm{A}^-\underset{k_{4r}}{\overset{k_4}{\rightleftharpoons}}\mathrm{P}^+|+\mathrm{A}^-, \tag{4}$$

$$\mathrm{P}^+|\,\mathrm{A}^-\overset{k_5}{\rightleftharpoons}\mathrm{P}|\,\mathrm{A}. \tag{5}$$

The vertical bar represents the lipid-water interface. The P| is the (surface) concentration of MgOEP at the interface and |A is the concentration of acceptor at the interface. Its relation to the bulk concentration will be discussed below.

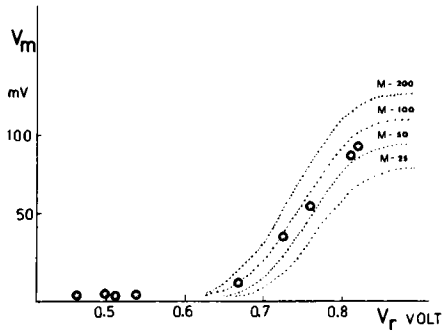

FIGURE 5

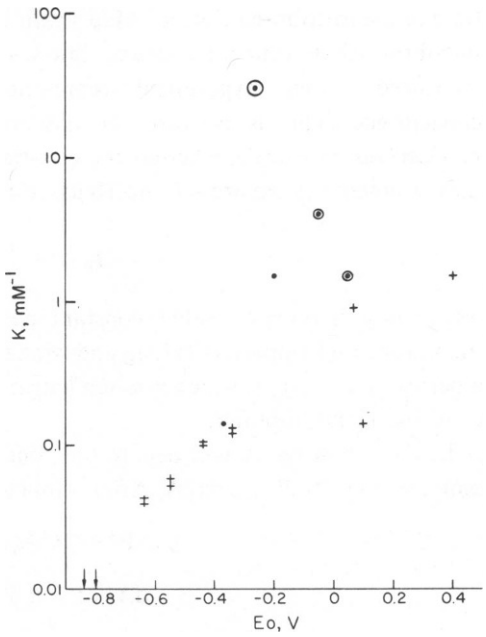

FIGURE 6

FIGURE 5 Membrane potential vs. redox potential of solution on one side of a MgOEP-containing membrane. The other side contained 2.5 mM ferrocyanide. If ferrocyanide was absent the membrane potential was considerably smaller. The curves represent solutions of the theoretical equation with V_0 = 0.8 V and M values as indicated on the curves. Decreasing V_0 would have the effect of shifting the curves to the left by the same amount as the change in the V_0, and vice versa.

FIGURE 6 The constant, K, for various acceptors is plotted on a log scale vs. the redox potential of the acceptors. Data come from Table I. The abbreviations are to be found in Methods. Viologens are plotted as double crossed, quinone disulfonates as points, quinone sulfonates as circled points, and inorganic ions as crosses.

Excitation of the porphyrin is given by $\theta = 1 - \exp(-\sigma E)$, where E is the light-pulse energy (in photons/cm^2) and σ is the optical cross section. The probability of intersystem crossing if the triplet state is reactive is included in θ. Eq. 3 represents the actual electron transfer reaction that occurs when $|A$ is within a critical distance, r_o, of $P^*|$. Our experiments in free solution show this center-to-center distance to be ~20 Å (Carapellucci and Mauzerall, 1975; Mauzerall, 1976; Ballard and Mauzerall, 1979). The interpretation is that the electron tunnels through 5–10 Å of inert solvent. By enforcing some separation of the donor and acceptor, the interfacial reaction favors ion formation and escape (Mauzerall and Hong, 1975). The complex $P^+|A^-$ either dissociates, k_4, or undergoes reverse electron transfer to the ground state, k_5. Although writing the dissociation and recombination as processes with simple first-order rate constants is an oversimplification, we are interested only in the yield of ions, which is in fact a constant determined by the properties of the particular system (Ballard and Mauzerall, 1980).

Under the conditions of the present experiments, where A^- concentration is low, the reverse association (k_4) can be neglected. This condition is easily verified by measuring the recovery

time of the membrane charge (Mauzerall, 1979). We also neglect the motion of P^+ across the membrane that occurs on a slower time scale (Hong and Mauzerall, 1976). The peak current measured in these experiments represents the stabilized charge, $P^+|$, on the membrane capacitance. This is because the electron transfer and dissociation (k_4) occur on the nanosecond time scale, whereas the duration of the light pulse and the time constant for the measurement system are ~1 and 10 μs, respectively. Thus:

$$I_P = \alpha \int_0^T (dP^+|/dt)dt \tag{6}$$

where α is a proportionality constant determined by the electrical characteristics of the membrane and amplifiers (Hong and Mauzerall, 1976; Mauzerall, 1979). The integral is over a period of $T \sim 10$ μs, which is much longer than the light pulse and is determined by the time constant of the amplifier.

In the following, X will denote the concentration of $P^+|A^-$, and the vertical bar will be omitted from $P|$, $P^*|$, and $|A$. After δ-function excitation

$$P^* = P_0^* \exp - (k_{2_r} + k_3A)t, \tag{7}$$

$$dX/dt = k_3P^*A - (k_4 + k_5)X, \tag{8}$$

$$dP^+/dt = k_4X, \tag{9}$$

and

$$I_P = \alpha \int_0^T (dP^+/dt)dt = [k_4/(k_4 + k_5)]\left[\int_0^T k_3P^*Adt - \int_0^T (dX/dt)dt\right]. \tag{10}$$

The second integral in Eq. 10 is zero, since the lifetime of X is $<T$. Because $k_{2_r} \gg 1/T$,

$$I_P = \alpha \frac{k_4P_0^*}{k_4 + k_5} \cdot \frac{k_3A}{k_{2_r} + k_3A}. \tag{11}$$

Eq. 11 shows the hyperbolic dependence of I_P on A with saturation peak current, I_{max}, as $A \longrightarrow \infty$, and K are given, respectively, by:

$$I_{max} = \alpha\theta P_0[k_4/(k_4 + k_5)], \tag{12}$$

and

$$K = k_3/k_{2_r}. \tag{13}$$

The term k_3 of Eq. 3 can be divided into two steps: the approach of A to within a critical distance from P^* at the interface and the actual electron transfer act. The latter is known to occur in <100 ns by direct measurement with ferricyanide at saturating concentration (Hong and Mauzerall, 1976). If the actual electron transfer act is extremely rapid, then k_3 will be diffusion limited, i.e., $\sim 10^{10} M^{-1}s^{-1}$. This constant is thus the upper limit of k_3. The lower limit of k_{2_r} is $\sim 10^7$ s^{-1} for a triplet state quenched by oxygen. Thus, the maximal value of K is ~1 mM^{-1}. Put simply, if both O_2 and the acceptor react with P^* at encounter-limited rates, their concentrations must be equal for competition to occur. If it is the singlet excited state that reacts, k_{2_r} is $\sim 10^9$ s^{-1}, and the corresponding maximal value of K would be ~0.01 mM^{-1}. In Table I it can be seen that for two acceptors, anthraquinone-2-sulfonate and phenan-

thraquinone sulfonate, the value of K exceeds even the upper limit for the triplet reaction. We conclude, therefore, that at least for these acceptors there must be accumulation of the acceptors at the membrane interface.

In general, if one assumes that the relation between the concentration at the interface, $|A$, and the concentration in the bulk is given by a constant partition coefficient, β, Eq. 13 should be modified to:

$$K = \beta k_3 / k_2, \tag{14}$$

It is possible therefore that a considerable part of the variation in K of the various acceptors does not reflect changes in the electron transfer rate but only differences in the degree of "accumulation" at the interface. One is tempted to ascribe the generally lower K for cationic acceptors (in comparision with anionic acceptors) to the finite angle between the zwitterionic dipole head of the lecithin molecule and the membrane surface (Stamatoff et al., 1979). This forms a dipole field with outside positive. Interestingly, the inner dipole field, estimated from the difference in anion and cation conductivity, is inside positive (Anderson and Fuchs, 1975; McLaughlin, 1977). In support of this distinction, we did not observe any effect of phloretin on the K of diquat or anthraquinone sulfonate (unpublished observation). Phloretin is known to induce a dipole field near the membrane interface, the inside of the membrane being more positive (Anderson et al., 1976). Thus, the interfacial electric field is quite complex and probably reverses itself twice. The electron transfer reactions might constitute a probe of the aqueous side of the interface, whereas ionic conductance measurements tend to reflect the situation at the lipid side of the interface.

The striking efficiency of anthraquinone sulfonate as compared with anthraquinone disulfonates (Table I) expresses the importance of orientation and approach of the hydrophobic moiety of the quinone at the membrane interface. The π system may be able to approach the pigment more closely, and yet not contact it. The effect of accumulation of neutral quinones at the membrane interface is analyzed in a separate communication (Krakover et al., 1981).

The variation of K in the four "viologen" acceptors used in this study probably reflects the variation in k_3, since it is plausible that their β are the same. The direction of the change tallies qualitatively with generally predicted relationships between the rate constant of a reaction and the change in standard free energy, ΔG, of the reaction (Rehm and Weller, 1970; Agmon and Levine, 1977; Scandola and Balzani, 1979), (Fig. 6). Failure to observe a decrease in k_3, and thus a decrease in K, with acceptors having positive redox potentials (i.e., for reactions having large negative ΔG) is not in agreement with the Marcus theory (Marcus, 1956; also see Rehm and Weller, 1970). It also seemingly contradicts the simple tunneling theory since the overlap between energy levels of the excited donor and vacant acceptor orbitals may be expected to decrease in this range (Gerischer, 1961). It should, however, be recognized that the energy level of the empty acceptor orbital estimated from the thermodynamic redox potential represents only the lower limit of vacant energy states. The electron can tunnel into higher levels, including those forbidden to electromagnetic radiation by dipole selection rules, and it can relax to the lower level on a picosecond time scale. The complexity of these molecules ensures a near continuum of acceptor levels (Mauzerall, 1978). Thus, simple tunneling cannot be excluded as a mechanism in the electron transfer processes studied in this paper.

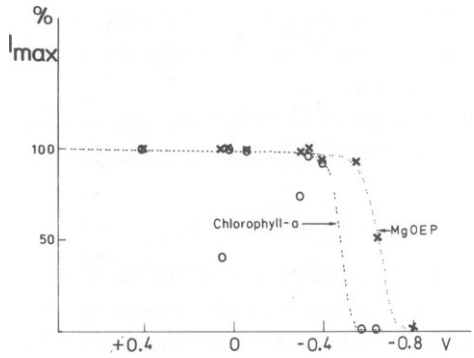

FIGURE 7 A plot of I_{max} for various acceptors as a function of their redox potential. ×, MgOEP membranes. O; chlorophyll *a* membranes. The points represent the following compounds from left to right: ferricyanide, hexa-aminoruthenium (III), NQS, PQS, AQS, diquat, paraquat, triquat, tetraquat, and cobalt hexacyanide.

It is also worthwhile to recall that the redox potential is a free energy level, whereas the energy overlap requirement for the tunneling process refers to internal energy. The difference between these two is the change in entropy that accompanies the redox process. Although much neglected, these entropic changes may be significant for large molecules having complex interactions with their environment (Gurney, 1936). They must certainly be taken into account when estimating the "efficiency" of photochemical reactions where potentials of products (a free energy) are compared with the energy of an excited state (an internal energy) (Ballard and Mauzerall, 1980).

The maximal electron transfer, I_{max}, will depend on the ratio of k_4/k_5 (see Eq. 12). The fact that I_{max} is independent of the nature of A below a cutoff level of redox potential (Fig. 7) implies that $k_4 > k_5$ for these acceptors. Conversely, for those acceptors which have lower I_{max}, the model suggests that k_5/k_4 increases. An increase in k_5 as the acceptor redox potential becomes more negative is indeed expected. According to the model suggested in this paper, however, a decreasing k_3 cannot account for a lower I_{max}. A zero I_P may indeed reflect a low k_3, since a reduced k_3 implies a low K, in which case the concentration range used in our experiments may have been too low to detect any current. Nevertheless, if current signals are observed at some concentrations, e.g., tetraquat-MgOEP and $Ru(NH_3)^{+3}_6$-Chl, the extrapolation to infinite concentration will yield a saturation current given by Eq. 12, i.e., independent of k_3. In agreement with our model, the low values of I_{max} for $Ru(NH_3)^{+3}_6$ and 2-AQS in Chl membranes would also be caused by an increase in the back reaction, k_5. This may be an oversimplification, as a more complex model would allow some admixture of electron transfer rate (k_3) into the empirical K. The situation is quite analogous to that of static and dynamic quenching of fluorescence.

APPENDIX

On the assumption of a constant field across a homogeneous membrane, the potential difference V_m, is given by (Goldman, 1943; Hodgkins and Katz, 1949):

$$V_m = (RT/F) \ln (\Sigma U_+ C^1_+ + \Sigma U_- C^2_-)/(\Sigma U_+ C^2_+ + \Sigma U_- C^1_-), \quad \text{(A1)}$$

where U_+ and U_- are the mobilities of monovalent cations and anions, respectively, in the membranes, and C^1 and C^2 are the concentrations in the membrane of these ions at interfaces 1 and 2, respectively. For the sake of this discussion it is assumed that except for the porphyrin cation P^+ the membrane is completely symmetric. If, therefore, the redox potential of the solution, V_r is much more negative than that of the porphyrin, V_0, V_m will be zero. If we denote numerator and denominator of Eq. A1 as $\Sigma U_i C_i$, and assume that V_r is kept sufficiently negative at interface 2, Eq. A1 will become

$$V_m = (RT/F) \ln (U_P C_{P^+} + \Sigma U_i C_i)/\Sigma U_i C_i. \quad \text{(A2)}$$

The concentration of the porphyrin cation at the membrane interface 1, C_{P^+}, is given by the standard Nernst equation:

$$\ln C_{P^+}/(C_{P_0} - C_{P^+}) = (F/RT)(V_r - V_0) = y, \quad \text{(A3)}$$

where C_{P_0} is the total prophyrin concentration in the membrane interface, which is assumed to be constant. From Eq. A3,

$$C_{P^+} = C_{P_0} e^y/(1 + e^y). \quad \text{(A4)}$$

If we introduce Eq. A4 into Eq. A2 and denote $U_P C_{P_0}/\Sigma U_i C_i = M$,

$$V_m = (RT/F) \ln [Me^y/(1 + e^y) + 1]. \quad \text{(A5)}$$

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REFERENCES

Agmon, N., and R. D. Levine. 1977. Energy, entropy, and the reaction coordinate: thermodynamic-like relations in chemical kinetics. *Chem. Phys. Lett.* 52:197–201.

Anderson, O. S., A. F. Finkelstein, I. Katz, and A. Cass. 1976. The effect of phloretin on the permeability of thin lipid membranes. *J. Gen. Physiol* 67:749–771.

Anderson, O. S., and M. Fuchs. 1975. Potential energy barriers to ion transport within lipid bilayers. *Biophys. J.* 15:795–830.

Ballard, S. G., and D. Mauzerall. 1979. Kinetic evidence for electron tunneling in solution. *In* Tunneling in Biological Systems. B. Chance and D. deVault, editors. Academic Press, Inc., New York. 581–589.

Ballard, S. G., and D. Mauzerall. 1980. Photochemical ionogenesis in solutions of zinc octaethyl porphyrin. *J. Chem. Phys.* 72:933–947.

Burnett, J. N., and A. L. Underwood. 1965a. Electrochemical reduction of 1-methyl-3-carbamidopyridinium chloride. *J. Org. Chem.* 30:1154–1158.

Burnett, J. N., and A. L. Underwood. 1965b. Electrochemical reduction of diphosphopyridine nucleotide. *Biochemistry.* 4:2060–2064.

Carapellucci, P. A., and D. Mauzerall. 1975. Photosynthesis and porphyrin excited state redox reactions. *Ann. N.Y. Acad. Sci.* 244:214–238.

Clark, W. M. 1960. Oxidation-Reduction Potentials of Organic Systems. Williams & Wilkins Co., Baltimore. 431.

Cleland, W. W. 1970. Steady-state kinetics. *In* the Enzymes. P. P. Boyer, editor. Academic Press Inc., New York. Chapter 1.

Davis, D. G. 1978. Electrochemistry of porphyrins. *In* The Porphyrins. D. Dolphin, editor. Academic Press Inc., New York. 5:127–152.

Felton, R. H. 1978. Primary redox reaction of metalloporphyrin. *In* The Porphyrins. D. Dolphin, editor. Academic Press Inc., New York. 5:53–135.

Fuhrhop, J. and D. Mauzerall. 1969. The one-electron oxidation of metalloporphyrins. *J. Am. Chem. Soc.* 91:4174–4181.

Gerischer, H. 1961. Semiconductor electrode reactions *In* Advances in Electrochemistry and Electrochemical Engineering. P. Delahay, editor. John Wiley & Sons, Inc., New York. 139–232.

Gill, R., and H. I. Stonehill. 1952. Polarographic investigation of the redox behavior of certain hydroxy- and sulfonic acid derivatives of anthraquinone. *J. Chem. Soc.* 1845–1847.

Goldman, D. E. 1943. Potential, impedance, and rectification in membranes. *J. Gen. Physiol.* 27:37–60.

Gurney, R. W. 1936. Ions in solution. Cambridge University Press, Cambridge.

Hanania, G. I. H., D. H. Irvine, W. A. Eaton, and P. George. 1967. Thermodynamic aspects of the potassium hexacyano-ferrate (III)-(II) system. II. Reduction potential. *J. Phys. Chem.* 71:2022–2030.

Handbook of Physics and Chemistry, 36th Edition. 1954. Chemical Rubber Company Publishers, Cleveland.

Hodgkin, A. L., and B. Katz. 1949. The effect of sodium ions on the electrical activity of the giant axion of the squid. *J. Physiol.* 108:37–77.

Hong, F. T., and D. Mauzerall. 1972. Photoemf at a single membrane-solution interface specific to lipid bilayers containing magnesium porphyrins. *Nature (Lond.).* 240:154–155.

Hong, F. T., and D. Mauzerall. 1976. Turnable voltage clamp method: application to photoelectric effects in pigmented bilayer lipid membranes. *J. Electrochem. Soc.* 123:1317–1324.

Hong, F. T. 1976. Charge transfer across pigmented bilayer lipid membrane and its interface. *Photochem. Photobiol.* 24:165–189.

Krakover, T. A., A. Ilani, and D. Mauzerall. 1980. The apparent inhibition of photoredox reactions of magnesium octaethyl porphyrin at the lipid bilayer-water interface by neutral quinones. *Biophys. J.* 35:93–98.

Marcus, R. A. 1956. On the theory of oxidation reduction reactions involving electron transfer. *J. Chem. Phys.* 24:966–978.

Masters, B., and D. Mauzerall. 1978. Effect of quinones on the photoelectric properties of chlorophyll-a containing lipid bilayers. *J. Membr. Biol.* 41:377–388.

Mauzerall, D. 1976. Electron transfer reactions and photoexcited porphyrins. *In* Chlorophyll-Protein Reaction Centers, and Photosynthetic Membranes. Brookhaven Symposium in Biology. 28:64–73.

Mauzerall, D. 1978. Electron transfer photoreaction of porphyrins. *In* The Porphyrins. D. Dolphin, editor. Academic Press, Inc., New York. 5:29–52.

Mauzerall, D. 1979. Photoinduced electron transfer at the water lipid-bilayer interface. *Life Sci. Res. Rep.* 12:241–259.

Mauzerall, D., and F. T. Hong. 1975. Photochemistry of porphyrins in membranes and photosynthesis. *In* Porphyrins and Metalloporphyrins. K.M. Smith, editor. Elsevier Scientific Publishing Co., Amsterdam. 701–725.

McLaughlin, S. 1977. Electrostatic potential at membrane-solution interfaces. *Curr. Top. Membr. Transp.* 9:71–135.

Meites, L. 1965. Polarographic techniques, 2nd edition. John Wiley & Sons, Inc., New York. 678–689.

Meyer, T. J., and H. Taube. 1968. Electron transfer reactions of ruthenium ammines. *Inorg. Chem.* 7:2369–2379.

Michaelis, L., and M. P. Schubert. 1937. Some problems in two-step oxidation treated for the case of penanthrene-quinone-sulfonate. *J. Biol. Chem.* 119:133–140.

Michaelis, L., and M. P. Schubert. 1938. The theory of reversible two-step oxidation involving free radicals. *Chem. Rev.* 22:437–470.

Milazzo, G., and S. Caroli. 1978. Tables of Standard Electrode Potentials. John Wiley & Sons, Inc., New York.

Mueller, P., D. O. Rudin, H. T. Tien, and W. C. Wescott. 1963. Methods for the formation of bimolecular lipid membranes in aqueous solutions. *J. Phys. Chem.* 67:534–535.

O'Reilly, J. E. 1973. Oxidation reduction potential of the ferro-ferricyanide system in buffer solutions. *Biochim. Biophys. Acta.* 292:509–515.

Rehm, D., and A. Weller. 1970. Kinetics of fluorescence quenching by electron and H-atom transfer. *Isr. J. Chem.* 8:259–271.

Scandola, F., and V. Balzani. 1979. Free energy relationship for electron transfer processes. *J. Am. Chem. Soc.* 101:6140–6142.

Stamatoff, J. B., W. F. Gaddick, L. Powers, and D. E. Moncton. 1979. Direct observation of the hydrocarbon chain tilt angle in phospholipid bilayers. *Biophys. J.* 25:253–262.

Turro, N. J. 1965. Molecular Photochemistry. W. A. Benjamin Inc., New York. 94.