ELECTRON TRANSPORT ACROSS GLYCEROL MONOOLEATE BILAYER LIPID MEMBRANES FACILITATED BY MAGNESIUM ETIOCHLORIN

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ABSTRACT The transport of electrons across biological membranes is believed to play an important role in many biophenomena. Although there have been many examples of systems which may be transporting electrons across Mueller-Rudin bilayer lipid membranes (blm), none has been well characterized. The system we describe here comprises a glycerol monooleate blm containing a magnesium etiochlorin (Mg-C) separating two aqueous phases each containing ferricyanide, ferrocyanide, KCl, and a platinum electrode. The E^0s for the $Mg\text{-}C^+/Mg\text{-}C$ and ferri-/ferrocyanide couples are 0.22 and 0.24 V vs. SCE. Thus the $Mg\text{-}C^+/Mg\text{-}C$ system is easily poised by the ferri-/ferrocyanide system. When the potentials of the ferri-/ferrocyanide couples are different on each side of the blm we show that the open-circuit membrane potential nearly equals the difference between the redox potentials. This is unequivocal evidence that electrons are being transferred across the blm from one aqueous phase to the other. On the basis of these experiments we deduce that electron transport is the major charge transport mechanism. When redox potentials are the same on each side of the blm, the conductance of the membrane can be $>10^{-3}$ S/cm^2. The conductance is proportional to the second power of the concentration of Mg-C in the membrane-forming mixture. A number of additional experiments are described which attempt to elucidate the mechanism of electron transfer. We believe that our data are consistent with the idea of an electron-hopping mechanism in which the transmembrane electron transport occurs by a series of second-order electron transfers between membrane-bound electron donors (Mg-C) and acceptors ($Mg\text{-}C^+$). Alternative explanations are presented.

INTRODUCTION

Electron transfer across biological membranes is believed to be important in many biophenomena. Lund's early work (1–3) suggested the association of redox phenomena and measured biopotentials and Mitchell's chemiosmotic hypothesis (4) continues to stimulate attempts to elucidate mechanisms of transmembrane electron transfer (5, 6). Many workers have attempted to demonstrate these phenomena using Mueller-Rudin-Tien-Wescott (7) bilayer lipid membranes (blm) doped with a variety of materials. Most of this work (8–11) describes attempts to observe photo-induced or photo-assisted electron transfer.

Szent-Györgyi (12) proposed the idea of conduction bands associated with energy levels in biological systems, thereby offering a mechanism by which "naked" electrons might translocate. Jahn (13) suggested that compounds such as β-carotene could bridge a membrane and offer a pathway for electronic conduction. In support of this idea, Ilani and Berns (14) and

Mangel et al. (15) have shown that β-carotene might act as a transmembrane "wire" facilitating the translocation of electrons. A recent study (16), however, has questioned the role of the β-carotene. Perhaps the best evidence for a "wire" is presented by the works of Dutton, Rentzepis, Parson, Fajer, Feher, and their respective coworkers (17–20) elucidating the transport of an electron in a photosynthetic reaction center. In *Rhodopseudomonas sphaeroides* for example, the electron translocates by a series of short distance electron transfers from an excited bacteriochlorophyll *a* dimer to a bacteriopheophytin *a* to a ubiquinone-iron complex to ubiquinone to a cytochrome *b*. Schönfeld et al. (20) have been able to incorporate these reaction centers into planar bilayers and demonstrate the photo-induced electron transfer. With this exception, most of the observed (putative) electron transport systems probably involve some sort of carrier mechanism (analogous to that proposed for actin or valinomycin mediated transport of alkali ions [21]) whereby the electron is translocated in association with a mobile membrane-bound redox couple which exchanges electrons (at the membrane-solution interfaces) with a redox couple in the aqueous phase. Masters and Mauzerall (22) have discussed a blm system containing membrane-bound chlorophyll *a* (Chl) with ferricyanide in one aqueous phase and ferrocyanide in the other. Photogenerated Chl^+ is the suggested current carrier. The observed photocurrents ($<10^{-10}$ A/cm^2) increase with the addition of membrane-bound quinones (Q). The behavior is consistent with a complicated carrier model invoking several mobile species: Chl^+, Q, and QH. The dark conductances in these systems are $<2 \times 10^{-7}$ S/cm^2 and the dark, open-circuit membrane potentials are <15 mV—considerably smaller than the several hundred millivolt difference expected between the half-cell potentials of the ferri- and ferrocyanide solutions.

Among the studies reporting electrical measurements on planar lipid bilayers, photocurrents have not been unequivocally demonstrated to be due to a translocation of an electron from one aqueous phase to another, although that is certainly a reasonable but not unique explanation for many of the reported phenomena. An excellent example of a system where the photo-induced current is not due to electron-transfer has been reported by Young and Feldberg (23), who demonstrate that the photocurrents result from the transport of H_3O^+ or OH^- effected by a photogenerated cation of magnesium octaethylporphyrin. Studies employing vesicles provide more direct evidence of electron transport across a lipid bilayer. Work by Hauska (24) demonstrates an electron translocation across a vesicular bilayer which may well be due to a membrane-bound plasto- or ubiquinone carrier. Photo-induced electron transfer across a vesicular bilayer has been demonstrated by many workers, e.g., Kurihara et al. (16) with chlorophyll *a* as the membrane-bound sensitizer and by Ford et al. (25) with Tris (2,2′-bipyridine) ruthenium (2+) as the membrane-bound sensitizer.

We report here on a blm system which facilitates efficient transmembrane electron transfer in the dark. The system may be schematically depicted:

$$Pt//A', B', KCl, buffer//GMO,Mg - C^+, Mg - C//A'', B'', KCl, buffer//Pt$$

The notations (′) and (″) indicate that the concentrations of redox components *A* (ferricyanide) and *B* (ferrocyanide) in the two aqueous phases are not necessarily identical. Magnesium etiochlorin (Mg-C) and its cation ($Mg\text{-}C^+$) are both present since the system is poised by the aqueous *A*/*B* couple.

The transport mechanism is examined using several electrical techniques: open-circuit

potential measurements in which the membrane potential is related to the difference in the redox potential of the A'/B' and A''/B'' couples; charge pulse measurements and constant current measurements in which the membrane conductance is measured in symmetrical systems (i.e., the compositions of the two aqueous phases are identical) as a function of the composition of the membrane forming mixture and of the potential of the A/B couple.

OPEN-CIRCUIT MEASUREMENTS

If a membrane selectively transports electrons it will exhibit a measurable open-circuit potential when the redox potentials of the A'/B' and A''/B'' couples on each side of the membrane are different. The total potential across the platinum electrodes separated by the selective membrane comprises the difference in the redox half-cell potentials plus the membrane potential:

$$\Delta E_{\mathrm{Pt,1}} = E_{A'/B'} - E_{A''/B''} + E_m. \tag{1}$$

The electrode immersed in the A''/B'' system serves as the reference. Electron transfer through the membrane between the A'/B' and A''/B'' couples will occur in the direction of the more positive couple as defined by the Nernst equation:

$$E_{A'/B'} = E^0_{A/B} + \frac{RT}{F} \ln \frac{[A']}{[B']} \tag{2}$$

and

$$E_{A''/B''} = E^0_{A/B} + \frac{RT}{F} \ln \frac{(A'')}{(B'')} \tag{3}$$

If the A'/B' couple is more positive, electrons will tend to move from the A''/B'' system across the membrane to the A'/B' system thereby creating an excess of negative charge on the A'/B' side of the membrane and an equal deficit of negative charge (excess of positive charge) on the A''/B'' side of the membrane. At equilibrium the potential of an ideally electron-selective membrane will be equal in magnitude and opposite in sign to the difference between $E_{A'/B'}$ and $E_{A''/B''}$:

$$E_{m,\mathrm{ideal}} = E_{A''/B''} - E_{A'/B'}. \tag{4}$$

If, however, ionic leaks partially dissipate the membrane potential then

$$E_m = \beta(E_{A''/B''} - E_{A'/B'}), \tag{5}$$

where

$$0 \leq \beta \leq 1. \tag{6}$$

The upper limit of unity for the value of β corresponds to an ideally electron-selective membrane while the lower limit of zero corresponds to a membrane where ionic transport processes dominate. The term β is not necessarily constant and may depend upon the solution compositions in some complex way.

Eqs. 1 and 5 may be combined:

$$\Delta E_{\mathrm{Pt},1} = (1 - \beta)\,(E_{A'/B'} - E_{A''/B''}). \tag{7}$$

When $\beta = 1$, $\Delta E_{\mathrm{Pt},1} = 0$. Experimentally, the value of β can be made equal to zero, or very nearly zero, simply by breaking the membrane. Then

$$\Delta E_{\mathrm{Pt},2} = E_{A'/B'} - E_{A''/B''}. \tag{8}$$

Thus, combining Eqs. 5 and 8 gives

$$-E_m = \beta\,\Delta E_{\mathrm{Pt},2}. \tag{9}$$

These equations ignore the contribution of liquid junction potentials arising from the asymmetry of the A', B', A'', and B'' concentrations.

Experimentally, as membranes are formed and broken, solutions on the (') and ('') side will mix through the membrane orifice (only when the membrane is broken). Thus if one begins with the condition where $[A'] = c$, $[B'] = 0$, and $[A''] = 0$, $[B''] = c$ the initial potential will be indeterminate (there is always some B' and A'' present). To a good approximation the amount of A' diffusing into the ('') solution and the amount of B'' diffusing into the (') solution will be equal. If, at any given time, an amount Δc_t has diffused, then

$$E'_{A/B} = E^0_{A/B} + \frac{RT}{F} \ln \frac{(A')}{(B')} = E^0_{A/B} + \frac{RT}{F} \ln \frac{c - \Delta c_t}{\Delta c_t} \tag{10}$$

and

$$E''_{A/B} = E^0_{A/B} + \frac{RT}{F} \ln \frac{\Delta c_t}{c - \Delta c_t}. \tag{11}$$

Thus (from Eq. 8)

$$\Delta E_{\mathrm{Pt},2} = 2\,\frac{RT}{F} \ln \frac{c - \Delta c_t}{\Delta c_t}. \tag{12}$$

and

$$E'_{A/B} = E^0_{A/B} + 1/2\Delta E_{\mathrm{Pt},2} \tag{13}$$

$$E''_{A/B} = E^0_{A/B} - 1/2\Delta E_{\mathrm{Pt},2} \tag{14}$$

Thus the individual potentials of the A'/B' and A''/B'' systems are easily approximated.

DETERMINATION OF MEMBRANE CONDUCTANCE USING CHARGE-PULSE AND CONSTANT CURRENT TECHNIQUES

In the charge pulse method (26–28) a charge is injected into the electrodes in <1 μs. This effects a voltage change across the blm which is observed at open circuit. Any phenomenon that facilitates charge transport across the blm will cause the membrane potential to decay. If these potential changes are small (< 0.025 V) and the relevant rate processes are at

steady-state the decay will be exponential (27, 28) i.e.,

$$V_t/V_0 = e^{-k_d t}, \tag{15}$$

and the usual semi-logarithimic plot, ln V_t vs. t, will have a slope of $-k_d$. It can also be shown that if the membrane-bound charges are immobile or if their number is small relative to the total charge passed during the decay (28), then

$$k_d = \frac{G_{ss}}{C_m}, \tag{16}$$

where G_{ss} is the steady-state conductance and C_m is the membrane capacitance. Since the charge injected, q, may be directly measured the value of C_m is experimentally determined:

$$C_m = \frac{q}{A_m V_0}, \tag{17}$$

where A_m is the area of the membrane and V_0 is the membrane potential at $t = 0$, immediately after charge injection. Thus

$$G_{ss} = \frac{k_d q}{A_m V_0}. \tag{18}$$

All the information required to estimate G_{ss} can be obtained from a single charge pulse experiment.

The conductance may also be determined using an applied constant current and measuring the steady-state change in voltage, V_{ss}:

$$G_{ss} = \frac{i_{ss}}{A_m V_{ss}}. \tag{19}$$

Combining Eqs. 17 and 18 gives

$$\frac{k_d q}{V_0} = \frac{i_{ss}}{V_{ss}}. \tag{20}$$

If this equality obtains, the assumptions that are prerequisite for Eq. 16 are valid.

The charge pulse method is also useful for deducing the properties of the "oil slick," i.e., the thicker barrier that is the precursor of the bilayer in the membrane-forming procedure. We are interested in the oil slick because its properties are more likely to be similar to the properties of the membrane-forming mixture. The thickness of the oil slick is of the order of several thousand Ångstroms as evidenced by the colors when it is viewed with white light. The variety of colors is indicative of uneven thickness over the surface. The electrical model for this system is greatly simplified if the following two assumptions are made: (*a*) the capacitance per unit area of the oil slick is inversely proportional to its thickness, i.e.,

$$C_{os} = \epsilon/\delta \tag{21}$$

and (*b*) the conductance per unit area of the oil slick is also inversely proportional to its thickness, i.e.,

$$G_{os} = g/\delta. \tag{22}$$

Thus the rate constant for open circuit decay of the charged capacitive element for a given region j will be

$$k_d = G_{\omega s,j}/C_{\omega s,j} = g/\epsilon, \tag{23}$$

whose value is independent of the thickness of the oil slick. In the absence of access resistance an oil slick comprising n regions of varying thickness can be modeled by n capacitances and n conductive elements in parallel and shown to be equivalent to a single parallel capacitive and conductive pair which has the same rate constant as each of the constituent regions. In the presence of an access resistance the possibility of a nonuniform voltage distribution during the injection of charge must be considered. The time constant for the charging process of a single region is easily estimated if the conductive element is ignored. Since the average capacitance per unit area of the oil slick is $\sim 1/70$ that of the bilayer, one can deduce that the time constant in 1 M KCl will be $<10^{-6}$ s. In the present study the time constant associated with rate constant k_d is considerably $>10^{-6}$ s and the access resistance may be ignored.

MATERIALS AND METHODS

Mg-C was synthesized by reacting excess Grignard reagent, EtMgI, with etiochlorin I (29–32) in ether. The resulting blue solution was combined with enough methanol to dispose of the excess Grignard and then evaporated to dryness. The residue was extracted with hexane and the hexane evaporated to give pure Mg-C. The purity was verified by taking the visible-UV spectrum in methylene chloride (Fig. 1). The peak positions and relative peak heights agree well with the values reported by Fuhrhop (33) for magnesium octaethylchlorin. The shape of the spectrum is quite similar to that reported for zinc octaethylchlorin (33) except that the Mg-C peaks at 404 and 618 nm are red-shifted from the corresponding Zn-C peaks by ~5 nm. There is a small absorption to the red of the 618 peak which may

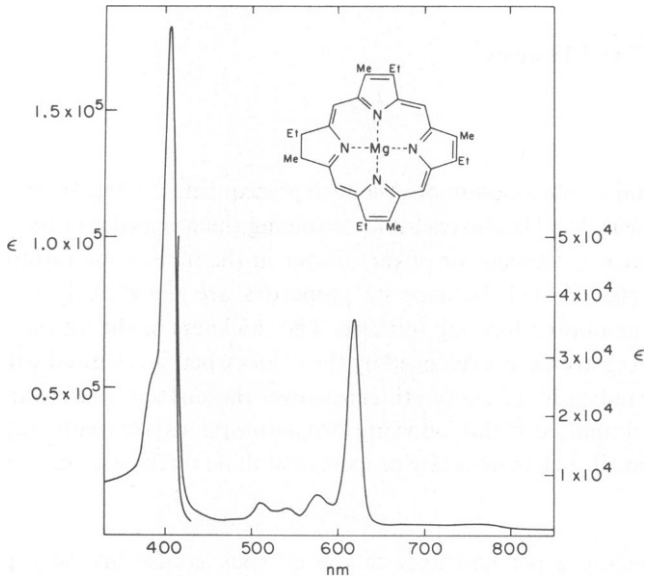

FIGURE 1 Spectrum of magnesium etiochlorin (1.42×10^{-5} M) in methylene choloride. Absorbance in the 650 to 800-nm region is probably due to a photooxygenation product (35).

correspond to a small amount (<2%) of impurity arising from photooxygenation (34, 35). This was not present in the freshly synthesized sample. Its presence did not appear to affect our results when comparisons were possible. Cyclic voltammetry in water-saturated methylene chloride with tetrapropyl ammonium perchlorate as supporting electrolyte (0.15 M) gives two reversible waves with E^0s of 0.22 and 0.66 vs. the saturated calomel electrode. These waves correspond to the oxidation of Mg-C to Mg-C^+ and Mg-C^+ to Mg-C^{++}. The difference between the two E^0s, 0.44 V, is less than the value of 0.49 V reported by Richardson et al. (36) for zinc etiochlorin or 0.48 V reported by Stolzenberg et al. (37) for zinc octaethylchlorin.

The E^0 for the ferri/ferrocyanide couple is 0.24 V, measured potentiometrically under the same experimental conditions used for bilayer formation (see below). This potential will vary with pH, ionic strength and potassium ion concentration.

Tetrapropylammonium perchlorate was prepared by neutralization of tetrapropylammonium hydroxide with perchloric acid in water. The precipitate was washed with water and recrystallized from ethanol.

The membrane-forming mixture was prepared from a stock mixture containing the solvent (hexadecane), and lipid (glycerol monooleate, 10–150 mg/cm^3). Weighed quantities of Mg-C were dissolved in measured volumes of the stock. Dissolution of the Mg-C was expedited by using methylene chloride which was then removed by gently purging the mixture with nitrogen. Dilutions were made from aliquots of the mixture and the stock. Manipulation of small volumes (<0.1 cm^3) was with SMI Micro/Pipettors (Scientific Manufacturing Industries, Emeryville, Calif.). Accuracy was verified by taking aliquots of the final preparation, diluting in known volumes of methylene chloride and noting the absorbance at 618 nm. This last procedure was not carried out routinely once the dependability of the preparative technique was established.

Using the pipette method of Szabo (38), planar black lipid membranes (7) were formed at room temperature (25 ± 1°C) across a 2-mm Diam hole in a teflon partition 0.025-cm^2 thick. The partition separates two teflon chambers containing various concentrations of KCl, potassium ferri- and/or ferrocyanide, and malate buffer (adjusted to pH 4.6). The total ionic strength was always close to 1.0. The cell design is typical (26) of that used for many blm studies. The electrodes were fabricated from Pt foil, ~2 cm^2. Membranes were observed and measured with a Wild stereoscope (Wild Heerbrugg, Farmingdale, N.Y.). The white, illuminating light was passed through two filters (Nos. 3387 and 5031, Corning Glass Works, Corning, N.Y.) thereby illuminating the membrane with light between 450 and 550 nm and avoiding the major adsorption peaks of the Mg-C at 617 and 400 nm. Aqueous phases were stirred with a magnetic stirrer and small magnetic stirring bars.

The charge pulser was designed and built by the Instrumentation Division, Brookhaven National Laboratory. The amount of charge injected during a given experiment could be measured using a calibrated capacitance in series with the cell. The FET voltage followers effected a factor of two amplification. This system was also used for following membrane potentials in open circuit experiments and in the constant current experiments. An excessively large charge pulse was used to break the membranes in the open circuit experiments.

A PAR programmer (Princeton Applied Research, Corp., Princeton, N.J.) in series with an appropriately selected resistance was used as a constant current source. The current applied to the membrane was deduced by subtracting the measured membrane potential from the applied voltage and dividing by the series resistance. The constant current was switched on using a reed relay.

Data were collected and stored with a Nicolet 1090 digital oscilloscope and Nic-283 tape coupler (Nicolet Instrument Corp., Madison, Wisc.) with a Kennedy 9700 tape drive (C. J. Kennedy Co., Altadena, Calif.).

The sequencing and timing of events (triggering of the oscilloscope, charge pulse, constant current) were accomplished with a home-built sequencer (Instrumentation Division, Brookhaven National Laboratory).

Racemic GMO was obtained from Supelco Inc., Bellefonte, Penns. Other chemicals were analytical or reagent grade and used without further purification. High-purity deionized (milli-Q) water was used throughout (Millipore Corp., Bedford, Mass.). All experiments were run at 25 ± 1°C.

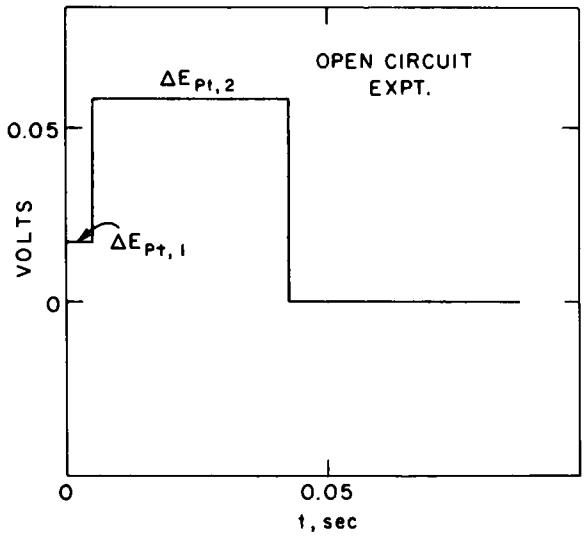

FIGURE 2 Open circuit experiment. GMO/*n*-hexadecane:: 25 mg/ml ≃ 0.07 M; [Mg-C] = 7.6×10^{-3} M. Initial concentrations of $A' = B'' = 1.06 \times 10^{-2}$ M; initial concentrations A'' and $B' \simeq 0$. Total malate concentration is 1.0×10^{-2} M adjusted to pH 4.6 with KOH; [KCl] = 0.98 M.

RESULTS AND DISCUSSION

Open-Circuit Experiments.

In these experiments the initial concentrations of A' and B'' were 1.06×10^{-2} M while the initial concentrations of A'' and B' were ~0.

The results of a typical experiment are shown in Fig. 2. As these experiments are repeated, components of the aqeuous phases diffuse through the barrier orifice (when the membrane or oil slick is not present) causing a continual diminution in the value of $\Delta E_{Pt,1}$ and $\Delta E_{Pt,2}$. (See Eqs. 10–14 and accompanying discussion.) At lower potentials the mixing process was enhanced by stirring. A plot of $-E_m$ (or $\Delta E_{Pt,2} - \Delta E_{Pt,1}$) vs. $\Delta E_{Pt,2}$ (Eq. 9) will have a slope β (Fig. 3). The plot is linear when $\Delta E_{Pt,2}$ is <0.11 V, indicating that β is constant in this region, with a value of 0.72. The fact that β is less than unity indicates there is some ionic transport process dissipating the potential. The open circuit experiment was also done without buffer and the results are virtually the same as shown in Fig. 3.

Conductance Experiments

Steady-state conductances of the bilayer lipid membranes and of the precursor oil slicks were measured using the charge pulse method. In some experiments the conductance of the blm was also measured using a constant current perturbation. In these cases the relationship in Eq. 20 obtained, indicating that intra-membrane charge translocation (really a displacement current) is an immeasurably small fraction of the total current passed during the charge pulse transient. It is also important to note that at a given concentration of Mg-C in the bilayer the dependence of conductance upon the concentration of ferri- and ferrocyanide is very slight when $A\ (=B) > 0.01$ M. When $A\ (=B) < 10^{-3}$ M there is a definite decrease in conductance

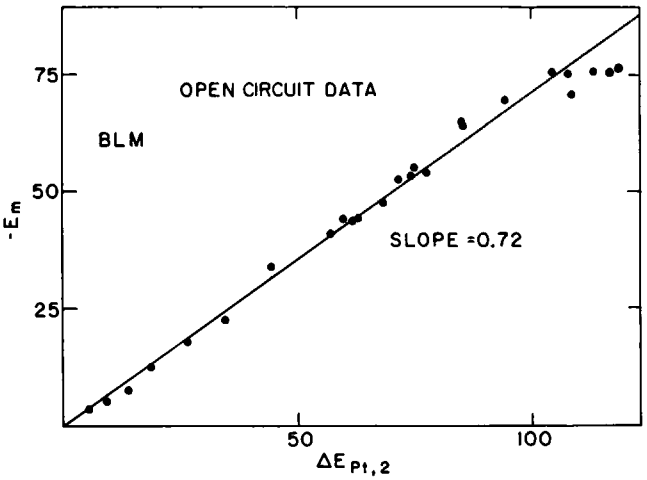

FIGURE 3 Plot of the (negative of) membrane potential ($-E_m$) vs. the difference in redox potentials of the A'/B' and A''/B'' systems ($\Delta E_{Pt,2}$). The E^0 of the ferri-/ferrocyanide couple under these conditions is 0.24 V vs. SCE. See Fig. 2 legend for details. Slope = 0.72. The potentials $E'_{A/B}$ and $E'_{A'/B}$ can be estimated using Eqs. 13 and 14.

as well as concentration polarization of A and B in the aqueous phases. Variations of pH (using malate buffer) between 4.6 and 5.2 or removal of buffer caused no perceptible changes in the data.

Assuming a value $C_m = 6.2 \times 10^{-7}$ F/cm^2 conductances are computed (see Eqs. 15 and 16) and plotted as log G_{ss} vs. the log of the concentration of Mg-C in the membrane-forming mixture (Fig. 4). The slopes of the log-log plots depend upon the concentration of GMO in the membrane-forming mixture. The limiting value of this slope appears to be ~2.

It is also possible to make charge pulse measurements on the oil slick and deduce the value of k_d. (Experiments were attempted in which k_d was measured on an oil slick containing only hexadecane and Mg-C—no GMO. These oil slicks were extremely unstable and only a few data were obtained. The oil slicks do conduct, however, indicating the GMO is not essential for conduction.) The dependence of k_d upon the concentration of Mg-C in the membrane-forming mixture is shown in Fig. 5. The slopes of these log-log plots also vary with GMO concentration and are virtually identical to the corresponding curves in Fig. 4. Equally interesting is that at a given concentration of GMO and Mg-C in the membrane-forming mixtures the values of the rate constant, k_d, for the oil slick and for the bilayer are surprisingly similar (simply divide G_{ss} [Fig. 4] by 6.2×10^{-7} farads/cm^2 to obtain k_d [Eq. 16]).

Conductance measurements of blm were also carried out in which the Mg-C concentration was maintained constant in the membrane-forming mixture while the GMO concentration was varied. A plot of log G_{ss} vs. log [GMO] is shown in Fig. 6. The slope is ~ -1.5 (the dotted line indicates a slope of -2.0). We believe that this simply indicates a change in the composition of the membrane-forming mixture which may modify the partitioning of Mg-C into the blm.

The conductance of the blm was also measured as a function of the redox potential of the A/B (ferri/ferrocyanide) system. The concentration of the Mg-C in the membrane-forming

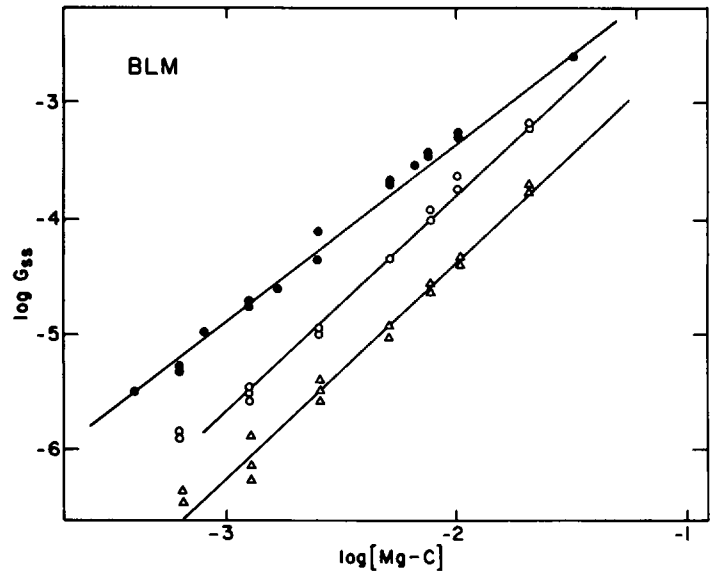

FIGURE 4 Plot of log of conductance of the bilayer (G_m) as a function of the log of the concentration of Mg-C in the membrane-forming mixture. For all cases: $[A] = [B] = 1.00 \times 10^{-2}$ M; malate = 0.01 M, pH 4.6; [KCl] = 0.98 M. The three data sets are for different concentrations of GMO in the membrane-forming mixture: GMO/*n*-hexadecane (slope):(●) 25 mg/ml or ~0.070 M (1.54); (○) 50 mg/ml or ~0.140 M (1.90); (Δ) 150 mg/ml or ~0.210 M (1.89).

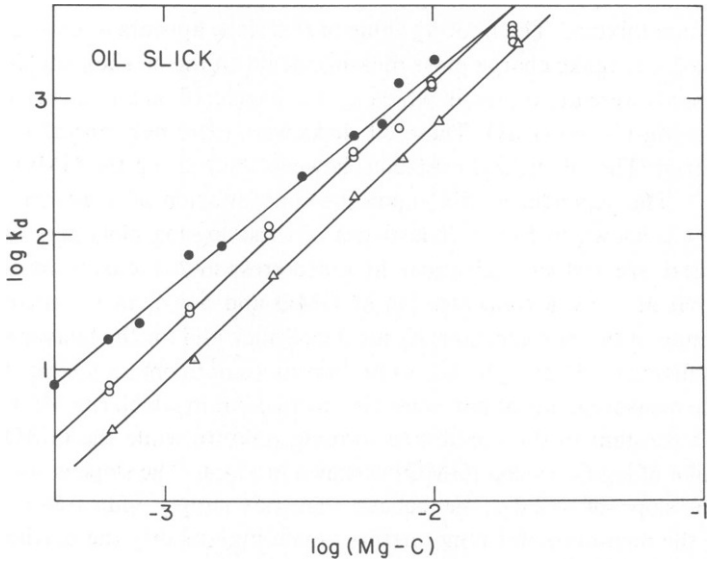

FIGURE 5. Plot of log of decay constant, k_d, for the oil slick as a function of the log of the concentration of Mg-C in the membrane-forming mixture. The three data sets are for different concentrations of GMO in the membrane-forming mixture (see Fig. 4 legend for details). Slopes:(●) 1.58; (○) 1.86; (Δ) 1.90.

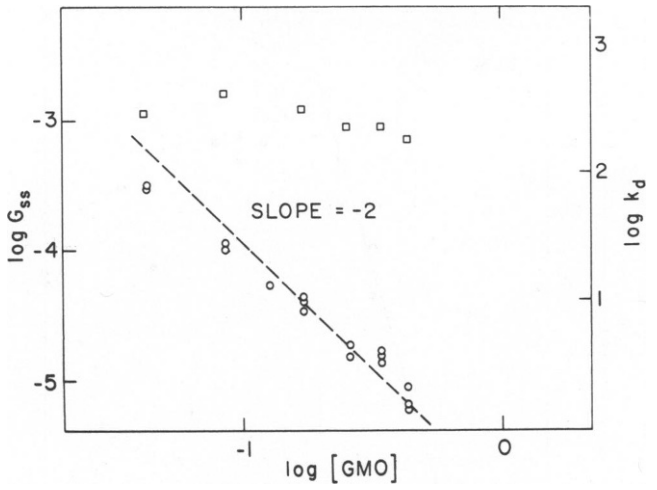

FIGURE 6 The log of the conductance, G_{ss}, of a bilayer (O), and the log of the decay constant, k_d, of an oil slick (□) as a function of the concentration of GMO in the membrane-forming mixture. Total malate = 0.01 M (pH = 4.6); $[A] = [B] = 0.01$ M; [KCl] = 0.98 M; [Mg-C] = 0.0049 M.

mixture was maintained constant. The results, Fig. 7, indicate that over a range of >100 mV the log of the conductance increases linearly with the potential with a slope = 1/0.059. We infer from this data that the concentration of the neutral Mg-C in the bilayer is maintained at a constant value during the formation of the bilayer while the concentration of the cation, Mg-C$^+$, increases linearly:

$$(\text{Mg} - \text{C}^+)_{\text{blm}} = (\text{Mg} - \text{C})_{\text{blm}} \exp\left\{\frac{F}{RT}(E_{A/B} - E^0_{\text{Mg-C}^+/\text{Mg-C}})\right\}. \tag{24}$$

If the conduction mechanism involves some sort of second-order interaction such as an electron transfer:

$$\text{Mg} - \text{C}^+ + \text{Mg} - \text{C} \overset{k_{ex}}{\rightleftarrows} \text{Mg} - \text{C} + \text{Mg} - \text{C}^+, \tag{25}$$

the relationship between the conductance and the redox potential and the nearly square dependence upon the Mg-C concentration are both explained. The rate of such a process will depend upon the concentration of the reactants, and the self-exchange rate, k_{ex}, for the reaction. Dahms (39) and Ruff (40) have shown that such a mechanism can enhance diffusion currents in aqueous polarography of high concentrations of a redox species. This mechanism can also explain the conductance of the oil slick. The conductance of the blm (and of the oil slick) exhibits a maximum at ~0.28 V (Fig. 7). Since the E^0 of the Mg-C$^+$/Mg-C system is -0.22 V vs. SCE (see subsequent discussion) the total concentration of Mg-C$^+$ and Mg-C in the bilayer (or in the surface regions of the oil slick) is significantly increased and may be reaching some limiting value. Whatever constraints govern this limit, the result could be that poising potentials >0.28 V induce a decrease in the Mg-C concentration rather than an increase in the Mg-C$^+$. This could lead to the observed decrease in conductance.

Implicit in Eq. 24 and the subsequent discussion is the assumption that both the Mg-C and

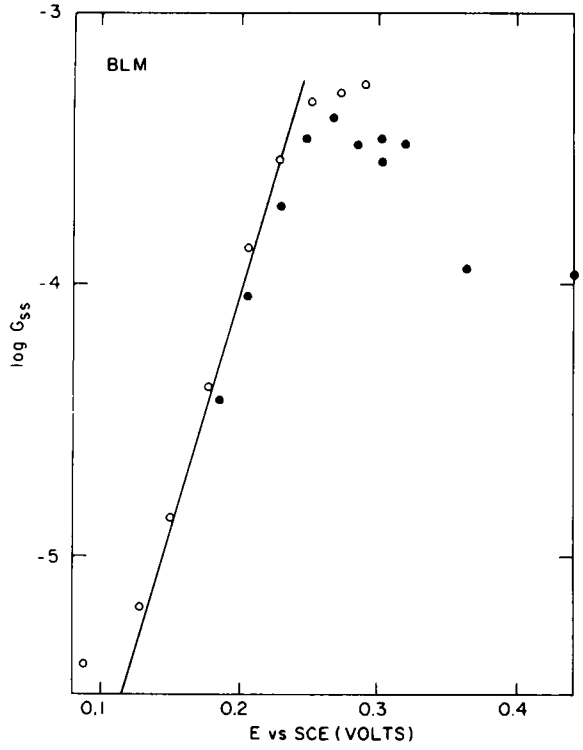

FIGURE 7 The log of conductance of a bilayer as a function of the redox potential of the A/B system. GMO/n-hexadecane: 25 mg/ml ≃ 0.07 M; total malate = 0.01 M adjusted to pH 4.6; ionic strength maintained ~1 M with KCl; [Mg-C] = 0.005 M. Case 1 (O) Initial [B] = 6.7×10^{-3} M with additions of A; Case 2 (•) Initial [A] = 6.7×10^{-3} M with additions of B. Solid line is drawn with theoretically expected slope (see text) of 1/0.059.

Mg-C$^+$ are stable in water. Smalley (private communication) has informed us that solutions of phosphatidylcholine vesicles containing Mg-C in 0.1 M KCl are stable for days. Introduction of ferri- and ferrocyanide to produce the cation indicates that the oxidation is reversible but that the cation degrades over minutes to hours. The E^0 of the Mg-C$^+$/Mg-C couple in vesicles was ~0.22 V vs. SCE as measured by cyclic voltammetry and by spectrophotometry with ferri-/ferrocyanide poising. This value agrees with the E^0 we measured in methylene chloride.

The question remains regarding the diminished power dependence of conductance upon the concentration of Mg-C at lower concentrations of GMO in the membrane-forming mixture. This may have something to do with the partitioning of Mg-C into the surface region of the oil slick and during the formation of the bilayer (remember that the behavior of the oil slick and the blm are similar). A preliminary experiment using bacterial phosphatidylethanolamine (BPE), n-decane, and Mg-C as the membrane-forming mixture gave results similar to those in Fig. 4 b and c: the slope of the log G_{ss} vs. log [Mg-C] was 2.0 (the conductances, however, were nearly two orders of magnitude lower for a given concentration of the Mg-C in the membrane-forming mixture). The slope of 2, however, for both BPE and GMO membranes would deem the explanation of unusual partitioning extremely unlikely.

CONCLUSIONS

The single unequivocal conclusion that we can reach based on the data is that GMO bilayers doped with Mg-C can transport electrons between redox systems in the separated aqueous phases. The value for β approaching unity (Fig. 3) indicates that electron transport is the major charge carrying process. The fact that β is slightly less than unity indicates that there must be some (as yet unidentified) ion transport occurring. Variation of pH (4.6–5.2) using malate buffer and the absence of buffer did not significantly change the results. Thus, the buffer components are not being transported across the membrane. Previous work (23) has shown that the cation of Mg-octaethylporphyrin can act as a carrier for hydroxide. It is possible that Mg-C^+ acts similarly; the absence of effects due to changes in pH or removal of buffer does not necessarily exclude this possibility.

The high steady-state conductances ($\sim 10^{-3}$ S/cm^2, Figs. 4 and 7) are orders of magnitude larger than any previously attributed to an electron transport mechanism in a bilayer. This may be due solely to the fact that the E^0s of the Mg-C^+/Mg-C and ferri-/ferrocyanide couples are well matched. It may also reflect some unique property of the Mg-C system such as high mobility in the bilayer and/or facile electron transfer (Eq. 25).

Our proposal that the electron transport involves a second-order electron transfer process (Eq. 25) must be viewed as speculative. A transport process in which a dimer (Mg-C—Mg-C^+) is the translocating species could also explain the (nearly) square dependence of conductance on the concentration of Mg-C. The mechanism would be analogous to that proposed to describe the behavior of weak-acid uncouplers (41–43) that induce a membrane conductance proportional to the square of the concentration of uncoupler in the blm.

The classical carrier mechanism (21) invoked by Masters and Mauzerall (22) cannot predict a second-order dependence. There remains the possibility that because of some unusual partitioning phenomenon the concentration of Mg-C in the blm (or in the surface regions of the oil slick) is related to the square of the Mg-C concentration in the membrane-forming mixture. Then, of course, the simple carrier mechanism could obtain. A direct measurement of the concentration of Mg-C in the bilayer could resolve this question, and we are now attempting to utilize the fluorescence of Mg-C as the basis of such a measurement.

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