

Charge Transfer Across a Single Lipid-Water Interface Causes Ion Pumping Across the Bilayer

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ABSTRACT The photoformation of magnesium-porphyrin cations (P^+) at a single lipid bilayer-water interface can pump lipophilic borate anions completely across the lipid bilayer and causes an actual reversal of the photovoltage. The system consists of a lipid bilayer containing magnesium octaethylporphyrin, an aqueous or interfacial electron acceptor on one side, and chloro- or fluoro-substituted tetraphenylborate in both aqueous electrolyte solutions. With 1- μ s pulsed illumination, an immediate positive photovoltage is observed, which decreases on the microsecond and millisecond time scales. On the time scale of seconds, as the P^+ cation concentration decays in reverse electron transfer, the voltage swings negative to a value almost equal to its initial value and finally decays with a half-time (~ 20 s) longer than the time constant of the system (~ 5 s). Thus, an ion gradient across the membrane is formed, trapped by the nonlinear relation between ion mobility and ion concentration. Continuous light illumination confirms that negative charge moves in the direction opposite that of the initial photoinduced electron transfer. Steady-state measurements indicate an ion pumping efficiency of $\sim 30\%$. This simple mechanism may be a progenitor of photobiological ion pumps.

INTRODUCTION

Transmembrane charge transport is a fundamental process in all biological systems. An ion gradient across the cell membrane appears to be a characteristic of all living cells, and the maintenance of such ion gradients requires some sort of ion pump. The most studied of these molecular pumps are the photodriven proton pumps: bacteriorhodopsin (Bamberg et al., 1993) and the photosynthetic electron transport system (Laverne and Junge, 1993). These biological pumps require proteins for their activity, and it has been assumed that transmembrane electron transfer photo-reactions are required for the photosynthetic system to pump ions across a bilayer. However, here we show that photodriven electron transfer between simple molecules across a single interface can drive hydrophobic ions completely across the bilayer with remarkable efficiency.

Previous experiments have shown that a lipid bilayer can be photocharged on the nanosecond time scale by electron transfer from excited magnesium-porphyrin in the bilayer to an electron acceptor in the aqueous phase (Woodle et al., 1987), and that it takes more than 0.1 s for the porphyrin cation (P^+) to cross the bilayer (Woodle and Mauzerall, 1986). The symmetric photoformation of P^+ cations in the lipid bilayer greatly increases the transmembrane ionic current carried by lipophilic anions such as tetraphenylborate ($TPhB^-$). This is termed the photogating effect (Drain et al., 1989; Drain and Mauzerall, 1992; Mauzerall and Drain, 1992; Sun and Mauzerall, 1996). The translocation of ions across the lipid membrane is thus markedly affected by the intramembrane ion interactions. In the present study, the

internal membrane potential is generated by asymmetric photoformation of the magnesium-porphyrin cations within the lipid bilayer. These experiments demonstrate photoinduced pumping of the borate anions completely across the lipid bilayer, even though the P^+ cation is formed at a single membrane interface. The photoinduced electrostatic fields in the membrane and the nonlinear photogating effect simultaneously contribute to the mechanism of transmembrane ion pumping.

EXPERIMENTAL

A detailed description of the materials and apparatus can be found elsewhere (Sun and Mauzerall, 1996; Mauzerall and Drain, 1992). Typically, the lipid solution consists of 18 mM diphytanoylphosphatidylcholine and 3.6 mM magnesium octaethylporphyrin in *n*-decane. The lipid membrane is formed in a 1.5-mm-diameter hole in a Teflon sheet symmetrically dividing a 4-ml polyethylene cell with glass windows. The bathing solutions on both sides contain 0.1 M NaCl and 0.01 M HEPES buffer (pH = 7.8). In the standard experiments, 0.3 mM to 0.5 mM sodium anthraquinone-2-sulfonate (AQS) is added to one side of the membrane as an electron acceptor after the membrane is formed. The substituted tetraphenylborate in ethanol solvent is then added symmetrically to bathing solutions on both sides of the membrane. The current is monitored by a fast operational amplifier (model 1021; Teledyne, Philbrick, MA) with a homemade feedback circuit of adjustable gain and time constant. The photovoltage is monitored with a Stanford Research Instruments SR560 low-noise preamplifier ($10^8 \Omega$ input impedance, 1 MHz frequency response). The confirming measurements for photovoltage are carried out with a Keithley 617 programmable electrometer with $10^{14} \Omega$ input impedance for the long time range (>0.01 s), and a homemade fast voltage preamplifier (model A250 from Amptek)

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with $10^{12} \Omega$ input impedance in the 1–200 μs time range, because the input impedance of SR560 preamplifier is less than that of the membranes under some conditions. The pulse of light at 590 nm with 1 μs full width at half-maximum is from a Candela SLL-250 flash-lamp pumped dye laser. Pulsed light of saturating energy (~ 1 mJ) is used to illuminate the membrane unless otherwise noted. A 300-W slide projector resulting in $\sim 130 \text{ mW cm}^{-2}$ light in the ~ 400 –600 nm range at the membrane is used to illuminate the membrane for the continuous light experiments.

RESULTS

The membrane system and probing methods have been schematically shown in a companion paper (figure 1 *B* in Sun and Mauzerall, 1996). The definition of the signs of voltage and current is important in describing the direction of charge movements within and across the lipid bilayer. Both the photovoltage and the photocurrent caused directly by the interfacial electron transfer from the excited porphyrin to the acceptor have a positive (+) sign under these experimental conditions. Note that the positive photovoltage drives negative charges from the acceptor side toward the opposite side, causing negative current.

Evidence for photoinduced ion pumping

Pulsed illumination

The photovoltage caused by the asymmetric photoformation of magnesium-porphyrin cations (P^+) in lipid bilayers in the absence of lipophilic ions shows a decay that is linear when plotted versus log time (curve *a* in Fig. 1), which is characteristic of a distributed reaction system (Liu and Mauzerall, 1985). There is only a small negative polarization at longer times. The addition of tetraphenylborate anions (TPbB^-) to the system has little effect on the decay of the photovoltage and on the polarization (data not shown). However, the presence of substituted tetraphenylborate anions in the lipid bilayer causes dramatic changes in the photovoltage decay trace (curves *b* and *c* in Fig. 1). The photovoltage now rapidly decays on the 10–200- μs time scale (Fig. 2). The most probable reason for these effects is polarization; some borate anions move from an interfacial position on the acceptor side into the bilayer on the 10–200- μs time scale, partially canceling the positive photovoltage. The voltage achieves a near-steady state on the 10–100-ms time scale (curves *b* and *c* in Fig. 1) because of equilibrium formation of ion aggregates and transmembrane diffusion of hydrophobic ions. This involves the facilitated movement of P^+ , as shown by the decreased lifetime of P^+ in the presence of electron donor on the side opposite the acceptor (Sun and Mauzerall, 1996).

The largest effect caused by the borate anions is the striking negative polarization on the time scale of seconds. The negative voltage (~ 2 mV; Fig. 1) for the systems with 4 μM p-TCPhB $^-$ or p-TFPhB $^-$ anions is ~ 10 -fold larger

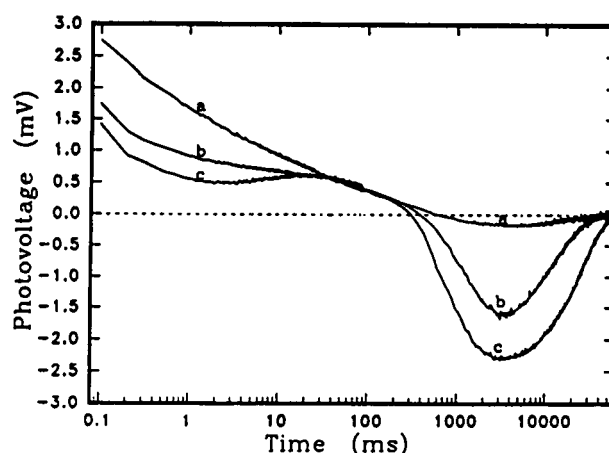


FIGURE 1 The photovoltage signals caused by the pulsed light are plotted versus log time. The voltage is measured using the amplifier with $10^8 \Omega$ input impedance. The lipid bilayer-forming solution contains 3.6 mM magnesium octaethylporphyrin. The bathing solution in each half-cell contains 0.1 M NaCl and 0.01 M HEPES at pH 7.8. AQS (0.3 mM) is added to one side of the membrane as an electron acceptor. These are the standard conditions. A 1- μs light pulse at 590 nm with signal saturating energy (~ 1 mJ) is used to illuminate the membrane. (a) No borate is present in the system. (b) 4 μM p-TFPhB $^-$ is added on both sides. (c) 4 μM p-TCPhB $^-$ is added on both sides.

than that for the system without borates. The negative voltage indicates that excess positive charges are left on the acceptor side, or that negative charges are left on the opposite side, after the disappearance of the P^+ . The latter

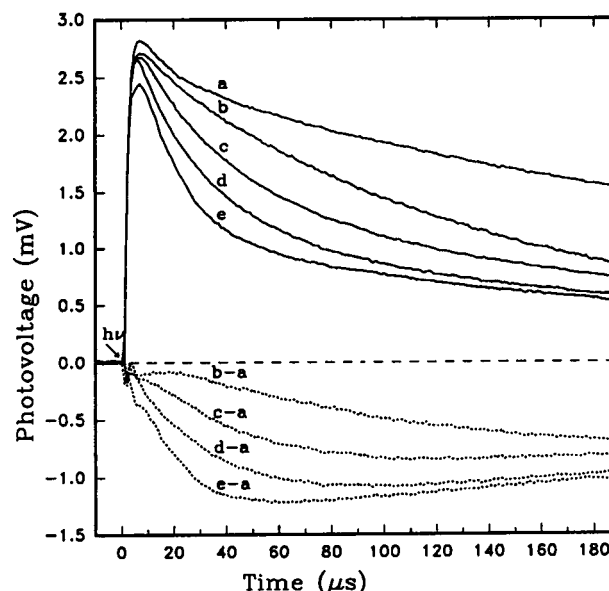


FIGURE 2 The rapid decay of photovoltage caused by borates is shown in the 1–200- μs time range. The voltage is measured using the fast preamplifier with a $10^{12}\text{-}\Omega$ input impedance. The conditions are the same as in Fig. 1. (a) No borate is present in the system. (b) 4 μM m-TFPhB $^-$. (c) 4 μM m-TCPhB $^-$. (d) 4 μM p-TFPhB $^-$. (e) 4 μM p-TCPhB $^-$. The dotted curves are the differences of the photovoltages between the borate-containing systems and the borate-free system.

conclusion is justified, because excess negative charges are photoformed on the acceptor side and only the negative ions are interfacially mobile. At equal concentrations of the different borates, the amplitude of the negative voltage decreases regularly in the sequence $p\text{-TCPhB}^- > p\text{-TFPhB}^- > m\text{-TCPhB}^- > m\text{-TFPhB}^- > \text{TPhB}^- \approx \text{no borate}$. This is also the sequence of enhanced conductivity or photogating effect by the P^+ cation (Sun and Mauzerall, 1996). This remarkable negative voltage is evidence for photoinduced electrostatic ion pumping. In a linear system, a photo or otherwise formed voltage across a membrane can decay by reverse reaction and/or by depolarization via ion movement into or across the membrane. The depolarization will only be appreciable if its rate equals or exceeds that of the reverse electron transfer reaction. However, in that case, reverse polarization occurs along with the slower reverse reaction, and no voltage reversal is possible. Reverse voltages can only be seen if the depolarization is faster than the reverse reaction and this reversal is faster than the repolarization, i.e., if the system is highly nonlinear. This occurs, for example, when the membrane capacitance is driven by a square-wave voltage pulse, the fall of which is driven faster than the system RC time (see below). In the present case, borate anions are pumped by the external and internal electric fields from the interface with the acceptor to the opposite interface with the conductance enhanced by P^+ (Drain et al., 1989). As the P^+ concentration decays on the time scale of seconds, the enhanced conductance vanishes. The negative voltage caused by the concentration gradient of transported borate anions then becomes visible, to be dissipated in turn on the long time scale. Stirring of the bathing solutions decreases the large negative polarization, as expected by dissipating the ion gradient in the aqueous phase. A strong argument that the borate anions have completely crossed the membrane is that the RC time constant for the membrane circuit is 5 s ($10^8 \Omega$ resistance, 50 nF capacitance), which is less than the observed decay time (~ 20 s). Thus the negative voltage on the long time scale must be caused by the Nernstian potential of the concentration gradient of borate anions across the membrane.

Support for this interpretation comes from adding the electron donor ferrocyanide on the same side as the electron acceptor to shorten the lifetime of the P^+ cations. This addition causes a faster decrease of the positive photovoltage and a correspondingly much smaller negative voltage (Fig. 3, *solid curve*) as compared to the $p\text{-TCPhB}^-$ system without ferrocyanide (Fig. 3, *dotted curve*). The decay of the photovoltage, in the presence of ferrocyanide, but in the absence of borates (Fig. 3, *dashed curve*) is more rapid still. Thus the presence of borate anions slows the reduction rate of the P^+ cation by ferrocyanide. This is expected from the rapid redistribution of P^+ across the membrane in the presence of borate anions (Sun and Mauzerall, 1996). In addition, the ferrocyanide causes an increased negative polarization on the fast time scale (Fig. 3, *dashed curve*), suggesting transient interfacial binding of this highly charged ion.

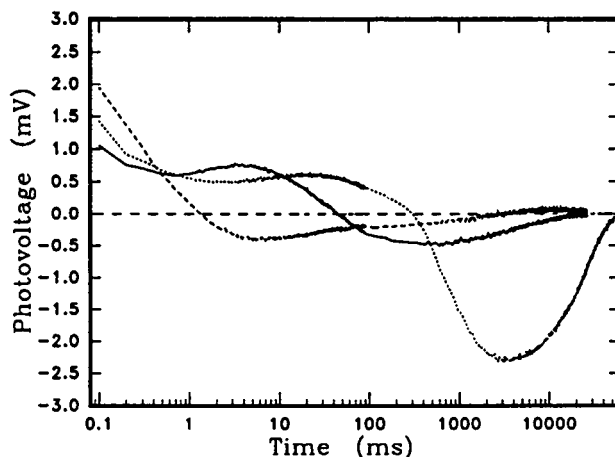


FIGURE 3 The photovoltages under various conditions are plotted versus log time. Conditions are as in Fig. 1. Dotted curve: $4 \mu\text{M } p\text{-TCPhB}^-$ is present without electron donor. Solid curve: $4 \mu\text{M}$ ferrocyanide is added to the same side as the acceptor in the presence of $4 \mu\text{M } p\text{-TCPhB}^-$. Dashed curve: $4 \mu\text{M}$ ferrocyanide is added to the same side as the acceptor in the absence of borates.

If donor is added to the side opposite the acceptor, a resurgence of positive voltage instead of a negative voltage is observed (figure 8 in Sun and Mauzerall, 1996). This resurgence occurs on the 10-ms time scale, which is the borate-induced transit time of the P^+ cation, and decays with the RC time of the membrane, ~ 5 s. The cause of the "new" positive voltage is the reaction of P^+ , which is made mobile by the borate anion (Sun and Mauzerall, 1996), with the donor at that interface producing transport of positive charges across the membrane in the same direction as the pumped borate anions.

Continuous illumination

Evidence for photoinduced ion pumping in the steady state is shown in Fig. 4. A large negative voltage (~ 10 mV) is observed after a small positive voltage transient (< 1 mV in the < 1 s time range) when continuous light illuminates the membrane in the presence of $4 \mu\text{M } p\text{-TCPhB}^-$ anion (Fig. 4, *curve b*). The negative voltage decreases in magnitude if the solutions on both sides of the membrane are stirred during the illumination. Only a small positive photovoltage (~ 2 mV in Fig. 4, *curve a*) is observed in the absence of borate anions. Thus the borate anions apparently invert the photovoltage. The negative voltage decays on the minute time scale, which also strongly supports the hypothesis that the borate anions have been pumped completely across the membrane. In contrast, about 60 mV positive voltage is recorded if 4 mM ferrocyanide (signal saturating concentration) is added to the solution on the side opposite the acceptor (Fig. 4, *curve c*). The increased translocation of P^+ allows electronic flow from donor to acceptor side to overtake the borate currents in the presence of donor (Sun and Mauzerall, 1996). The voltage swings negative after turning off the light, revealing the borate ion gradient. The system

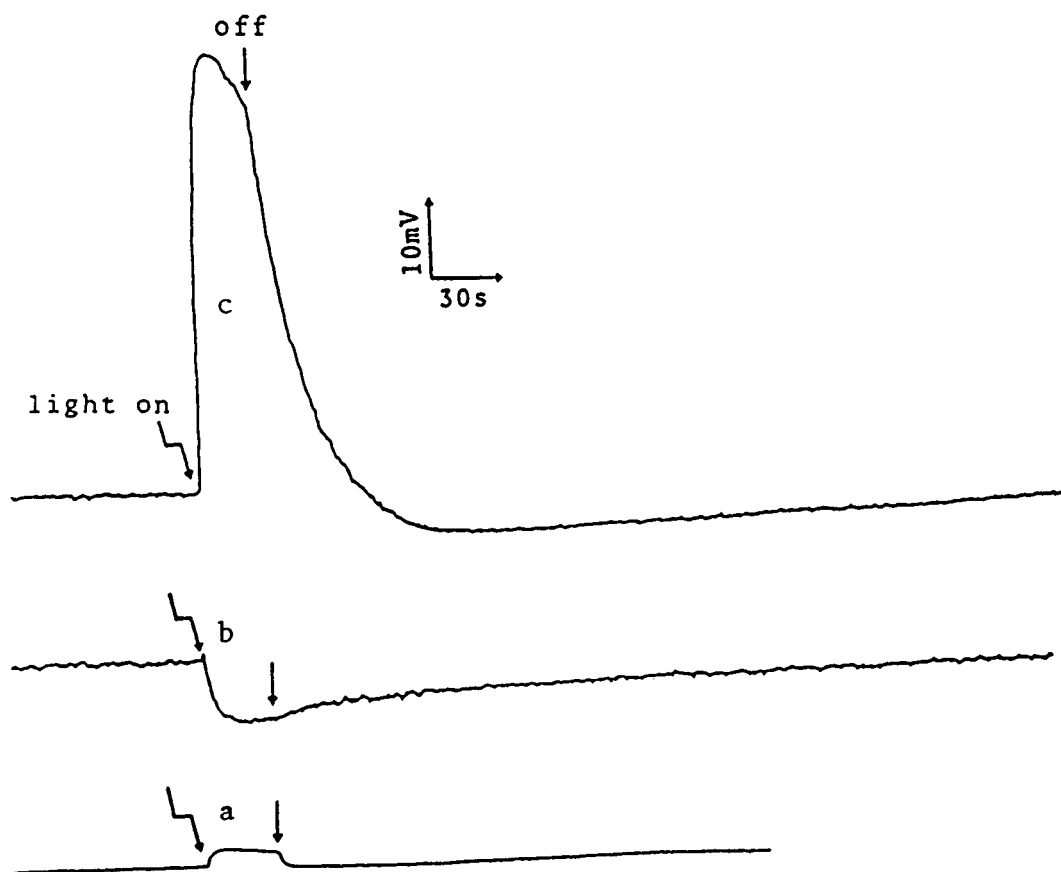


FIGURE 4 The voltages caused by continuous illumination of the membrane system are plotted versus time. The white light intensity at the membrane is $\sim 130 \text{ mW cm}^{-2}$. AQS (0.5 mM) is added to one side as an electron acceptor. (a) Neither borates nor electron donor is present. (b) $4 \mu\text{M}$ p-TCPbB $^{-}$ is added symmetrically to the system in the absence of donor. (c) 4 mM ferrocyanide is added on the opposite side of the acceptor in the presence of p-TCPbB $^{-}$.

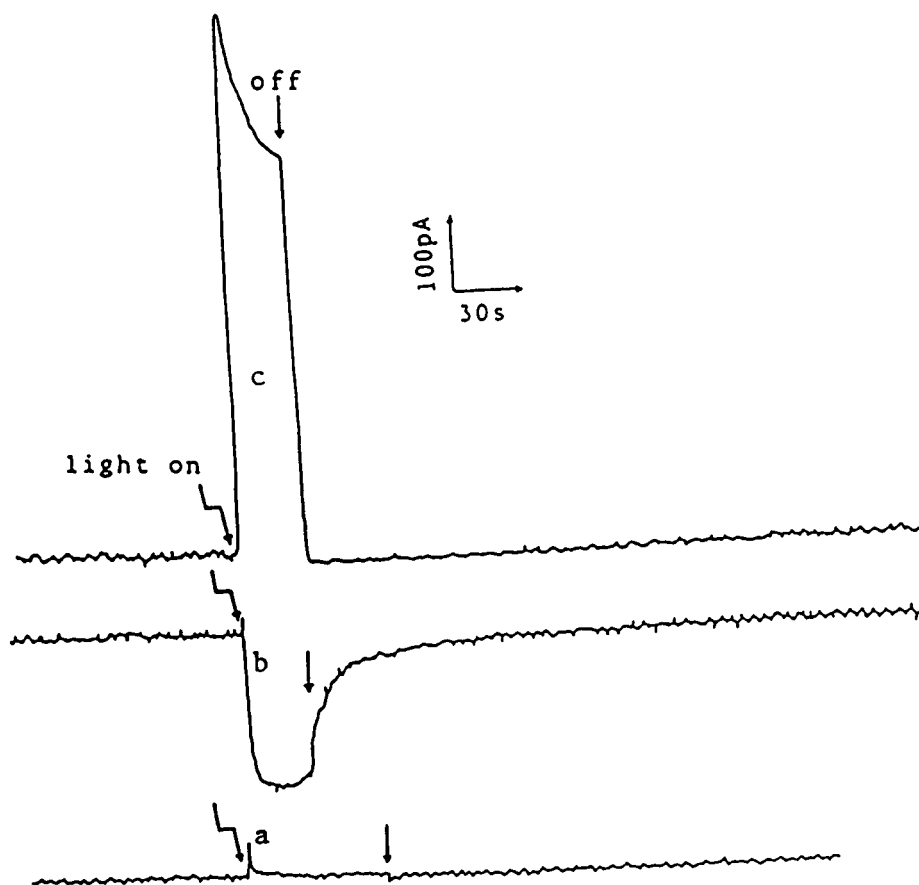
with the donor but without borates shows a similar phenomenon and a $\sim 30\%$ less negative voltage. This negative polarization becomes smaller with shorter illumination. Reillumination causes a photovoltage similar to that caused by the initial illumination. These phenomena are explained by photodriven movements of porphyrin cations and borate anions. The charge transferred by interfacial electron transfer (positive voltage) exceeds that of the borate transport (negative voltage), but the former decays with the RC time of the system, whereas the latter, Nernstian, voltage decays on a much longer, diffusive time scale.

Current measurements with continuous illumination confirm the concept of photoinduced electrostatic pumping of borate anions across the membrane. About 170 pA of negative current appears after a fast positive current transient when the membrane system, without electron donor, is illuminated with continuous light (Fig. 5, curve *b*). The half rise time of the negative current is on the ~ 500 -ms time scale, but that of the positive current of the system with ferrocyanide on the side opposite the acceptor (Fig. 5, curve *c*) is almost the same as the opening time of the shutter (~ 2 ms). When the continuous light is turned off, the decay of the negative current of the donor free system is also much

slower than that of the positive current of the system with the donor on the side opposite the acceptor. Stirring of the bathing solution has little influence on the decay of the currents. These widely different response times reflect the much faster transport of electrons than of ions when a *trans* donor is present (Sun and Mauzerall, 1996). The negative current shown by curve *b* in Fig. 5 indicates that the borate anions move from the acceptor side to the opposite side. This builds up the observed negative voltage on the long time scale in the open-circuit experiment (Fig. 4, curve *b*).

Fig. 6 shows the change of steady-state photoinduced current with increasing concentration of ferrocyanide on the side opposite the acceptor. In the presence of ferrocyanide at $>0.3 \mu\text{M}$ concentration, the positive current caused by transmembrane P^{+} -mediated electron transfer exceeds the negative current of ion pumping. The ratio of negative to positive limiting currents, 0.28, is the efficiency of the ion pumping (see Discussion). The measured current should be the summation of the electron transfer current (I_e) and the negative ion pumping current (I_p). If a constant I_p remains in the presence of ferrocyanide, the change of the total current (I_t) with the concentration of donor (C_d) should be fit by the equation $I_t = I_{\text{max}} a C_d / (1 + a C_d) + I_p$, derived by steady-

FIGURE 5 The currents caused by continuous illumination of the membrane system of Fig. 4 are plotted versus time. No voltage is applied across the membrane. The conditions are the same as in those in Fig. 4. (a) Neither borates nor electron donor is present. (b) $4\ \mu\text{M}$ p-TCPhB⁻ is added in the absence of donor. The half-decay time of the reversed current is within 2–3 min under illumination. (c) 4 mM ferrocyanide is added on the opposite side to the acceptor in the presence of p-TCPhB⁻.



state kinetics (see Appendix). However, the measured currents (Fig. 6, *filled circles*) cannot be fit by the calculated curve using this equation (Fig. 6, *dotted curve* versus *filled circles*). A good fit (Fig. 6, *solid curve* versus *filled circles*) can be obtained only when the value of I_p is inversely proportional to the concentration of donor. Under steady-state conditions, the current is proportional to the concentration of transported P^+ at the interface near the donor $[P^+]_d$ and is derived as $I_p = b/(1 + \alpha C_d)$ (see Appendix). The decrease of $[P^+]_d$ by the presence of donor has two effects on ion pumping: first, the internal ion pumping field is weakened, and second, the enhanced photogating effect on the anion conductance is decreased. The data listed in Table 1 demonstrate that the steady-state photogating effect on the membrane conductance of p-TCPhB⁻ is decreased over 10-fold in the presence of donor. The photogating effect can be calculated from the ratio of the photoinduced increase of the transmembrane current (driven by applied voltage) to its dark value (Drain et al., 1989). Because the enhanced currents of the asymmetric systems shown in Table 1 are not completely symmetric for opposite signs of applied voltage, the current differences between the -40 and $+40$ mV applied voltages can be used to calculate the photogating effect. In the absence of donor, this enhanced gating effect is ~ 30 -fold $((5100 + 5200) - 300)/300$, whereas in the presence of donor, it is only $\sim 75\%$ $((840 -$

$310) - 300)/300$). Thus, the striking decrease of the enhanced photogating effect on conductivity slows the ion pumping across the bilayer. This proves that the nonlinear enhanced conductivity makes an important contribution to the observed photoinduced ion pumping.

DISCUSSION

Mechanism of electrostatic ion pumping

What is the driving force?

Information on the mechanism of the photoinduced ion pumping can be obtained by comparing the amount of charge movement within the system. The measured voltage of -2 mV in Fig. 1 corresponds to ~ 100 pC with a membrane capacitance of ~ 50 nF. The measured potential difference across the membrane during the "driving" phase (~ 0.5 s duration of positive voltage for the borate free system; Fig. 1, *curve a*) averages at most 0.6 mV (the value of voltage at the 50-ms time scale). The average conductivity in the $4\ \mu\text{M}$ p-TCPhB⁻ system in the same time range is ~ 100 nS (Sun and Mauzerall, 1996), so the total charge transferred by the overall potential difference across the membrane can be only ~ 30 pC. This value is only $\sim 30\%$ of that calculated from the measured voltage. Thus the simple membrane outer voltage and conductivity cannot account

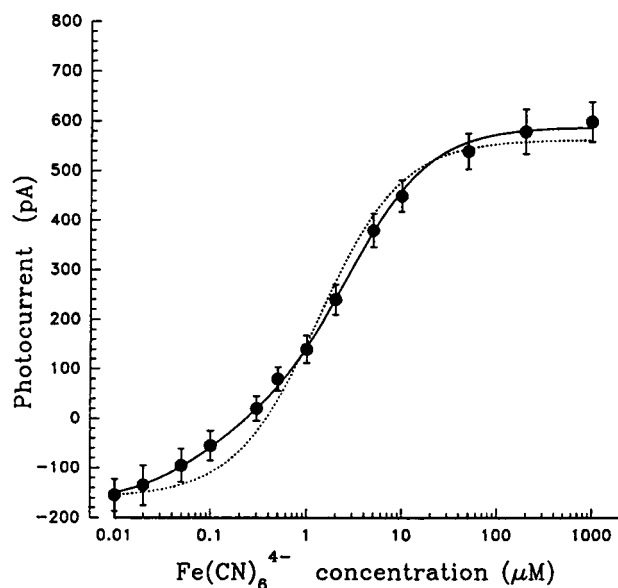


FIGURE 6 The steady-state current caused by continuous illumination of the system of Fig. 5 *b* is plotted versus log concentration of electron donor on the side opposite the acceptor. The conditions are same as those in Fig. 4. Filled circles are the measured current data. Dotted curve: $I = 556C_d/(1 + 0.76C_d) - 170$. Solid curve: $I = 215C_d/(1 + 0.36C_d) - 170/(1 + 11.6C_d)$.

for the number of the ions moved. In fact, the negative external (Nernstian) voltage is simply not present in the "short circuit" or voltage-clamped current measurements. In the "open circuit" voltage measurements, the ion pumping builds up a "DC" negative external voltage (Fig. 4, curve *b*) because no current flows. Thus, the driving force of the ion pumping is present inside the bilayer or at the interfaces. Our calculations from the exponential dielectric model (Mauzerall and Drain, 1992) show that the internal and interfacial fields, caused by the ions inside the lipid bilayer, can exceed applied fields by well over an order of magnitude. Astumian, Robertson, and Tsong (Astumian and Robertson, 1989; Tsong, 1990) have reported that a large local field within the membrane can be formed by a modest, externally applied AC field, and this can cause a transport enzyme to pump ions through the membrane against an (electro) chemical potential. In the present system, the large interfacial field produces a deceptively small external field,

but it is the motive force for the ion pumping. As in the AC field system, it is the nonlinear effects that lead to rectification and efficiency of the ion pumping.

What makes the reversed voltage observable?

Because the borate anions are mobile they can adjust their concentrations and positions to minimize their total energy by canceling internal fields. Only the field caused by the intramembranal P^+ and the interfacial A^- (reduced acceptor) remains because the movements of P^+ into the aqueous phase and of A^- into the membrane are negligible. The ion pumping shows that this field built by the photoreaction cannot be completely neutralized by the movement of B^- anions into that interface and into the membrane. The spilling over of this excess intramembranal B^- into the opposite aqueous solution constitutes the ion pumping. The voltage measured between the aqueous phases can be changed from the initial positive photovoltage to negative when sufficient borate anions are pumped to the side opposite the acceptor. The internal field direction may remain the same, driving the borate anions across the membrane in the same direction and building up the negative voltage until the internal field is neutralized by the opposing field across the membrane. The ion flux will be proportional to the amount of P^+/A^- present. This agrees with the observation of less ion pumping if the donor is added to either side of the membrane (Figs. 3 and 6). In fact, the quantitative fit to the data in Fig. 6 requires that the negative pumping current decrease as the inverse of the donor concentration (see Appendix).

Because of the symmetric, spatially variable dielectric coefficient of the membrane and the discrete charges inside this region (Mauzerall and Drain, 1992), the electrostatic fields in similar but opposite regions inside the membrane are expected to have opposing direction and differing magnitudes, even though the overall transmembrane voltage is positive. The transport rates of P^+ cations and p-TCPbB $^-$ anions across the membrane are increased by ~ 100 -fold when both are present, so that the P^+ can reach the *trans* interface on the 1–10-ms time scale (Sun and Mauzerall, 1996). This is important to the photoinduced electrostatic ion pumping process, because the rapid distribution of P^+ can contribute to the internal pumping fields in the same direction as the positive photovoltage. Moreover, the distribution of P^+ in the bilayer enhances the photogating effect on the membrane conductivity to borate anions by forming an ion chain or aggregate (Sun and Mauzerall, 1996). The average conductance of p-TCPbB $^-$ anions across the membrane is increased to ~ 100 nS in the 0.1-s time range after the photoformation of P^+ . The conductance returns to ~ 4 nS, which is the value in the dark, when all of the P^+ cations are neutralized on the ~ 1 -s time scale. The decay of this photogating effect is what traps the pumped anions at the other interface and makes them visible in the pulsed photovoltage measurements (Fig. 1).

TABLE 1 Comparison of the dark and photo currents caused by applied voltage with given sign on the opposite side the acceptor, 0.5 mM AQS, and by continuous illumination on the bilayer in the absence and presence of a *trans* electron donor

Applied voltage (mV)	No donor		2 mM $\text{Fe}(\text{CN})_6^{4-}$	
	I_{dark} (pA)	I_{photo} (pA)	I_{dark} (pA)	I_{photo} (pA)
0	<1	-160	<1	+580
-40	+150	+5100	+150	+840
+40	-150	-5200	-150	+310

Efficiency of electrostatic ion pumping

The efficiency of ion pumping can be taken as the ratio of charge pumped across the membrane to the charge photoformed in the membrane, which acts as the driving force for the mobile ions. The efficiency can be estimated for both the steady state and the pulsed illumination. For the steady state the ratio of the limiting negative pumping current to the positive current in the presence of saturating donor on the *trans* side (Fig. 6) is 0.28. This is a good approximation to the full efficiency, differing only by the differing gradient of P^+ at the donor interface in the two conditions.

For the pulsed case, the charge pumped across the membrane is ~ 100 pC, estimated from 2 mV on 50 nF (Fig. 1), but the charge as driving force cannot be simply calculated in the same way (Hong and Mauzerall, 1976). The reason is that the P^+ cation is formed within the membrane, not on its surface. The "equivalent circuit" view is that the membrane behaves as three capacitors connected in a series. The photoformed charge is on a large interfacial capacitor (unknown) across the polar region, not that externally measured across the membrane. The charge crossing the membrane on the pulsed illumination is increased to ~ 300 pC from 100 pC by the *trans* addition of saturating donor (figure 4, A and B, in Sun and Mauzerall, 1996). This shows that the P^+ cations carrying 200–300 pC of charge have crossed the membrane and reacted with the ferrocyanide. If 300 pC is the total photoformed P^+ , the efficiency is 33%. Thus, the efficiency of the ion pumping is $\sim 30\%$.

When the other borates are used instead of the p-TCPbB $^-$ or p-TFPbB $^-$ in the same system, the efficiency is much lower than the above value (data not shown). For the 4 μ M TPbB $^-$ system, the efficiency of the ion pumping is at most 1–2%. Thus a small change of structure has a large effect on the ion movement, supporting a cation-anion interaction model.

CONCLUSION

Lipophilic borate anions can be pumped across a lipid bilayer by the asymmetric photocharging of the membrane when the P^+ cations of lipophilic magnesium-porphyrin are photoformed at a single membrane-solution interface. The mechanism is very different from that found in biological ion pumps that involve proteins and pigment isomerization (Bamberg et al., 1993) or transmembrane electron transfer (Lavergne and Junge, 1993). The efficiency of the electrostatic ion pumping is $\sim 30\%$ for the p-TCPbB $^-$ anion, which is remarkable for a purely electrostatic system. The photoformed internal electrostatic field is the driving force for the ion pumping across the lipid bilayer. The drastic photogating effect of the membrane conductance greatly increases the transport rate and efficiency of the ion pumping.

Because the ion pumping system does not require proteins, but only lipophilic anions and cations, it is an excellent candidate for a prebiological ion pump. Liposomes are

readily formed under primitive earth conditions (Deamer et al., 1993), and given the specificity of many ion carriers, the present system would establish differing inner and outer environments. This is a crucial first step on the path to a living cell.

APPENDIX: STEADY-STATE KINETICS

The steady-state kinetics can be much simplified because the rapid transient internal movements of the ions are not relevant. Moreover, the continuous light intensity is not saturating, and to observe the effect of a *trans* donor, all other parameter (e.g., borate) concentrations are fixed. Because we are measuring the total steady-state current, one can focus on the interfacial charge transfer, and the interior of the membrane can be considered to be a stirred pot. In the steady state the rate of change of P^+ in the membrane is

$$dP^+/dt = \sigma IAP - (k_A + k_D D)P^+ = 0 \quad (1)$$

$$\text{with } P_0 = P + P^+,$$

where σ is the effective optical cross section for formation of P^+ from P in light of intensity I , A is the concentration of acceptor, k_A is the rate of constant for the reverse reaction of P^+ with adventitious reductant at the acceptor interface, and k_D is that for the reaction of P^+ and added donor (D) at the *trans* or donor interface.

Thus in the steady state,

$$P^+ = \sigma IAP / (\sigma IA + k_A + k_D D). \quad (2)$$

The flux of borate ions will be determined by the electric fields and concentration gradients at each interface. With the standard approximation of small fields, the fields and thus the flux of borate anions will be linear in P^+ . Thus the borate flux into the membrane at steady state will be

$$dB_i/dt = C(KB_0 - B_i) + MP^+KB_0 + C(KB_0 - B_i) = 0, \quad (3)$$

where B_i is the borate concentration in the membrane and B_0 that in the aqueous solution; K is the binding constant of the borate to the lipid bilayer; C is the interfacial diffusion constant, and MP^+ contains the interfacial mobility M and the electric field between A^- and P^+ , and thus is proportional to P^+ for a given distribution of P^+ . The first two terms represent the concentration gradient and electric field fluxes at the acceptor interface, and the last term is the gradient flux at the donor interface where the field is negligible. Thus in the steady state,

$$B_i = \frac{MP^+ + 2C}{2C} KB_0 \quad \text{i.e., } B_i > KB_0. \quad (4)$$

This gradient feeds the borate flux across the membrane. We calculate only the initial flux. With time, gradients will form in the aqueous layers, causing the fluxes to decrease and making them sensitive to stirring.

The current across the donor interface is

$$I/F = k_D DP^+ + C(KB_0 - B_i) = k_D DP^+ - MP^+ KB_0 / 2 \quad (5)$$

where F is the Faraday constant. Writing out these two terms:

$$I/F = \frac{\sigma IAP_0 k_D D}{\sigma IA + k_A + k_D D} - \frac{\sigma IAP_0 M KB_0}{2(\sigma IA + k_A + k_D D)}, \quad (6)$$

which are of the form used to fit the data in Fig. 6. The positive (electronic) current shows hyperbolic saturation with D, whereas the negative (borate) current is simply inversely proportional to D. The simplifying assumption of a borate flux linear in P^+ (Eq. 3) results in the same denominator for

both terms. A nonlinear dependence of the borate flux on P^+ will give differing saturation factors, as observed.

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REFERENCES

- Astumian, R. D., and B. Robertson. 1989. Nonlinear effect of an oscillating electric field on membrane proteins. *J. Chem. Phys.* 91:4891–4901.
- Bamberg, E., H.-J. Butt, A. Eisenrauch, and K. Fendler. 1993. Charge transport of ion pumps on lipid bilayer membranes. *Q. Rev. Biophys.* 26:1–25.
- Deamer, D. W., E. H. Mahon, and G. Bosco. 1993. Self-assembly and function of primitive membrane structures. In *Early Life on Earth*. Nobel Symposium 84. Columbia University Press, New York. 107–125.
- Drain, C. M., B. Christensen, and D. Mauzerall. 1989. Photogating of ionic currents across the lipid bilayer. *Proc. Natl. Acad. Sci. USA.* 86: 6959–6962.
- Drain, C. M., and D. Mauzerall. 1992. Photogating of ionic currents across the lipid bilayers: hydrophobic ion conductance by an ion chain mechanism. *Biophys. J.* 63:1556–1563.
- Hong, F., and D. Mauzerall. 1976. Tunable voltage clamp method: application to photoelectric effect in pigmented bilayer lipid membranes. *J. Electrochem. Soc.* 123:1317–1324.
- Lavergne, J., and W. Junge. 1993. Proton release during the redox cycle of the water oxidase. *Photosynth. Res.* 38:279–296.
- Liu, T. M., and D. Mauzerall. 1985. Distributed kinetics of decay of the photovoltage at the lipid bilayer-water interface. *Biophys. J.* 48:1–7.
- Mauzerall, D., and C. M. Drain. 1992. Photogating of ionic currents across lipid bilayers: electrostatics of ions and dipoles inside the membrane. *Biophys. J.* 63:1544–1555.
- Sun, K., and D. Mauzerall. 1996. Evidence for ion chain mechanism of the non-linear charge transport of hydrophobic ions across lipid bilayers. *Biophys. J.* 71:3323–3336.
- Tsong, T. Y. 1990. Electrical modulation of membrane proteins: enforced conformational oscillations and biological energy and signal transductions. *Annu. Rev. Biophys. Biophys. Chem.* 19:83–106.
- Woodle, M., and D. Mauzerall. 1986. Photoinduced ion movements in bilayer membranes containing magnesium octaethylporphyrin. *Biophys. J.* 50:431–439.
- Woodle, M., J. W. Zhang, and D. Mauzerall. 1987. Kinetics of charge transfer at the lipid bilayer-water interface on the nanosecond time scale. *Biophys. J.* 52:577–586.