

Fast and Efficient Charge Transport across a Lipid Bilayer Is Electronically Mediated by C₇₀ Fullerene Aggregates

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Abstract: Fullerene anions, made by photoreduction in a lipid bilayer, produce the largest trans-membrane steady state photocurrents yet observed, $\sim 6.0 \mu\text{A}/\text{cm}^2$. Since these photocurrents are not light saturated, their maximum value is considerably larger. Dithionite was used as electron donor for its ability to reduce photoexcited fullerenes at the donor interface on a time scale faster than 15 ns. Both photovoltage and photocurrent increase 15-fold on adding the acceptor ferricyanide trans to the donor. There are two components to the transit time of negative charge across the bilayer, $< 100 \text{ ns}$ and $6 \mu\text{s}$, in the 100 mM dithionite|0.6 mM C₇₀|5 mM ferricyanide system, where | stands for the water–bilayer interface. This is strong evidence that the conduction is electronic and not diffusive-ionic. The plot of the ratio of photovoltage for the dithionite|C₇₀| system to that of the dithionite|C₇₀|ferricyanide system versus concentration of C₇₀ in the lipid-forming solution is highly nonlinear. This suggests that aggregates of the fullerene are responsible for the fast negative charge transport. The action spectrum of the photocurrent further supports the existence of photoactive C₇₀ aggregates in the lipid bilayer. These aggregates may form the conductive path for electrons across the lipid bilayer.

Introduction

Electron transport across lipid membranes is a fundamental property of living systems and is exemplified in photosynthesis. The designs of molecular systems for solar energy conversion are mimics of this vectorial photoinduced charge transportation.^{1,2} However, relatively few synthetic molecules have been shown to transport charge across a lipid bilayer efficiently.^{3–5} With the recent success in producing macroscopic quantities of C₆₀ and C₇₀ fullerene molecules, many properties of these supermolecules have been determined. Since the photophysics of fullerene molecules resembles that of porphyrins and the fullerenes have low reduction potentials,^{6,7} they are expected to have interesting charge-transfer chemistry in both the ground state and the excited states. Electron transfer from various amines^{8,9} and semiconductor colloids¹⁰ to photoexcited singlet and triplet C₆₀ has been reported. Previous work in our laboratory has shown that both C₆₀ and C₇₀ embedded within a lipid bilayer can act as efficient electron acceptors from electron donors at the interface.¹¹ Further, the fullerenes can also efficiently transport negative charge completely across the

membrane.¹² However, the mechanism of electron transfer across the planar lipid bilayer and the limits to its maximum rate were unknown. The electron donors used in our previous work were either amphoteric zinc deuteroporphyrin or ascorbate. The interfacial reaction rates are 6 and 60 μs for deuteroporphyrin and ascorbate, respectively.¹¹ Since this is the time range in which ionic diffusive and electronic conduction can be separated,¹³ a faster interfacial reaction was desired. Using dithionite ion as electron donor, the interfacial electron transfer time was found to be $< 15 \text{ ns}$.¹³ Therefore, it was possible to study the transit time of charge transfer across the lipid bilayer and the mechanism of charge transfer. Herein, we report the photovoltage and photocurrent measurements on the new systems and present evidence that the charge transfer is most likely by electronic conduction mediated by fullerene aggregates.

Experimental Section

A DPPC (1,2-diphytanoyl-*sn*-glycero-3-phosphocholine, Avanti Polar-lipids, Inc.) planar lipid bilayer was formed across a 0.8 mm diameter hole in a thin Teflon sheet which divided two aqueous compartments.¹⁴ The photovoltage across the lipid bilayer has been measured with platinum electrodes and a differential amplifier (Tektronix Model P6046, 1 M Ω , 100 MHz) and recorded on a digital oscilloscope (Tektronix TDS540). The data were transferred to a HP Vectra PC-308 computer for analysis. The positive electrode was on the donor side. The transmembrane photocurrent has been measured using two calomel electrodes (KCl-agarose bridges, $R = 200 \Omega$) clamped at 0 V by an operational amplifier (time constant = 50 ns). The input electrode was on the acceptor side. When a voltage was applied, the stated sign was on this side. It must be noted that the voltage measurements in the asymmetric systems are only possible because of the limited impedance (1 M Ω) of the voltage amplifier. The RC time of the system is 4 ms. If a high-impedance ($> 100 \text{ M}\Omega$) amplifier is used, the dark voltage rises to $> 100 \text{ mV}$ with the asymmetric system dithionite|C₇₀|ferricyanide and breaks the membranes. Thus the voltage measurements are carried out on partially polarized systems. The

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Table 1. Photoelectric Properties of C₇₀ in a DPPC Bilayer Membrane with Dithionite as Donor and Different Acceptors^a

acceptor	—	AQS ¹⁻	Fe(CN) ₆ ³⁻	MV ²⁺	S ₂ O ₄ ²⁻
pulsed light					
V _{max} ^{fast} , mV	1.7	3.2	19.0	3.7	
t _{1/2,rise} ^{fast} , μs	0.015	0.015	0.015	0.015	
V _{max} ^{slow} , mV	1.1	2.1	13.3	2.6	
t _{1/2,rise} ^{slow} , μs	30	30	30	30	
I _{max} ^{fast} , mA ^b	30	160	420	400	390
t _{1/2,decay} ^{fast} , μs ^c	0.1	0.1	0.1	0.1	0.1
I _{max} ^{slow} , μA	<0.02	0.17	0.32	0.18	0.15
t _{1/2,decay} ^{slow} , μs			6		7
continuous light					
I _{dark} , nA	<0.01	0.5	1.6		0.8
I _{light} , nA	2.3	3.0	20.0		1.5

^a The bilayer forming solutions contain 0.6 mM C₇₀ (saturated) and the concentration of donor dithionite is 100 mM (saturated) for all systems. The acceptor solutions of AQS⁻, Fe(CN)₆³⁻, and MV²⁺ are saturated at 0.3, 5, and 25 mM, respectively. The 7 ns width pulsed light is at 532 nm and saturated at 0.1 mJ. The continuous light is 30 mW/mm² at 532 nm, not saturating. ^b These currents contain a contribution from the interfacial displacement current: $C dV/dt$. ^c The time constant of the system is 0.1 μs because of the access resistance and membrane capacitance.

current measurements are under "short circuit" conditions (voltage clamp at zero voltage) and so are responses to the polarized state. The pulsed illumination is from a Continuum Nd:YAG laser, Surelite 10 (0.1 mJ incident on the bilayer, pulse width 7.5 ns, 1 Hz), at a wavelength of 532 nm, except as otherwise specified. The continuous excitation at 532 nm is from an arc-lamp excited Nd:YAG Laser. The dependence of steady-state photocurrent on wavelength was measured by using a xenon arc lamp with interference and blocking filters as the light source. Intensities were determined with a Sci Tech thermopile. The bilayer-forming solution contains 0.6 mM C₇₀ (Materials and Electrochemical Research Corp.) for the standard experiments. C₆₀ was also used with similar results, but the stronger absorption of C₇₀ in the visible region resulted in larger signals. Sodium dithionite (Fisher Scientific, purified grade) was added to the positive electrode side of the aqueous compartment at 100 mM, and potassium ferricyanide (5 mM, Mallinckrodt, Inc.), the electron acceptor, was added to the trans compartment as specified. Solutions were deoxygenated with glucose-glucose oxidase.¹⁵

Results and Discussion

The major results of the photovoltage and photocurrent measurements are summarized in Table 1. Dithionite, S₂O₄²⁻, was selected as donor, and several acceptors, anthraquinone 2-sulfonate (AQS⁻), methyl viologen (MV²⁺), and ferricyanide (Fe(CN)₆³⁻), were utilized. Since the most efficient acceptor is ferricyanide in both pulsed and continuous excitation experiments, the discussion below will focus on the results with the standard system, 100 mM dithionite|0.6 mM C₇₀|5 mM ferricyanide, all at saturating concentrations. Data with other donors and the above acceptors can be found in our previous publication.¹³

(1) Photovoltage Measurement. The photovoltage at the donor interface (V_d) in the dithionite|C₇₀| system shows a rise time of <15 ns (Figure 1a). The compartment with dithionite is positive showing that the fullerene is reduced. On adding ferricyanide to the opposite (trans) side as acceptor, the photovoltage dramatically increased by a factor of 15, but the rise time remains the same as before (Table 1 and Figure 1b). A component of somewhat less amplitude with a rise time of 30 μs (Table 1) similarly increases on adding trans acceptor. The constancy of both the decay time and the ratio of its amplitude to that of the fast signal (0.68 ± 0.02) for all acceptors

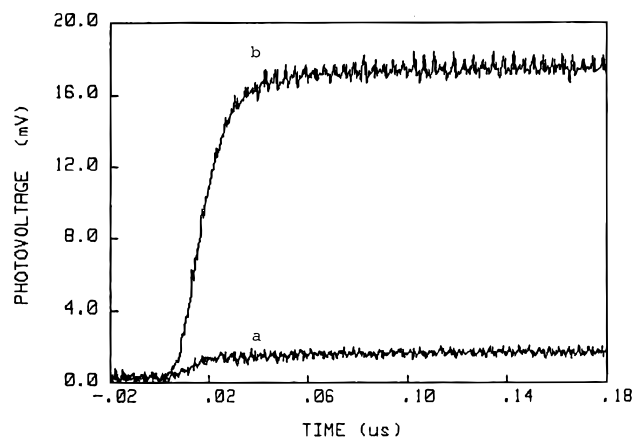


Figure 1. Photovoltage rise across a planar lipid bilayer. The bilayer forming solution contains 0.6 mM C₇₀. (a) Sodium dithionite is added to the positive electrode side at 100 mM. (b) Ferricyanide (5 mM) is added to the trans compartment of (a). The aqueous phase consists of 1 M NaCl, 10 mM Hepes, pH 8.3, and is deoxygenated enzymatically.¹⁵ The excitation wavelength is 532 nm (100 μJ, 7.5 ns, 1 Hz). Each trace is an average of 32 pulses. A lipid bilayer containing C₆₀ yields a similar result.

(Table 1) suggests that this component is a voltage-induced polarization, possibly at the Pt electrodes, since it is not seen in the voltage clamped current measurements. The photovoltage at the acceptor interface (V_a) was measured as a control in the |C₇₀|ferricyanide system in the absence of dithionite. V_a shows a slow rise with 30-μs half-time, with a maximum amplitude <0.5 mV. This is about 40 times less than the photovoltage amplitude when both acceptor and donor are present. Thus V_a contributes very little to the total photovoltage and a fast transfer of charge across the bilayer is apparently responsible for the large increase in photovoltage.

To study the dynamics of the donor interface reaction, the photovoltage rise time was measured as a function of the concentration of dithionite. Detectable photovoltage (0.1 mV) appears at a dithionite concentration of 5 mM. The rise time of the photovoltage is <15 ns even at this weak, initial signal. Increasing the concentration of dithionite to 100 mM only increases the amplitude of both the fast and the slow photovoltages with a half saturation of ~30 mM, but does not change their rise times. This result is in strong contrast to the dynamic reaction kinetics usually observed in similar systems.¹⁴ The common explanation of static kinetics is a preformed complex of reactants, but considering that the dithionite ions are in the aqueous solution and the fullerene molecules are in the lipid membrane, it is hard to imagine that complexes can be formed between them. One possible explanation is that the fast photovoltage rise is caused by the excitation of dark reaction generated fullerene anions, C₇₀⁻, driving electrons further into or even across the membrane. The second and third reduction potentials of fullerenes in CH₂Cl₂ are $E^0_{C_{70}^0/C_{70}^{2-}} = -0.76$ V_{SCE} and $E^0_{C_{70}^{2-}/C_{70}^{3-}} = -1.17$ V_{SCE}, respectively,⁷ so further reduction of C₇₀⁻ on excitation is possible. However, the steady-state photocurrent action spectrum shows strong evidence against activity of C₇₀⁻ (see below and Figure 5). This indicates that the photoionization of C₇₀⁻ or C₇₀²⁻ is not the reason for the photovoltage rise time of 15 ns. Dithionite ion dissociates into the highly reactive SO₂⁻ ion radical ($K = 1.4 \times 10^{-9}$ M¹⁶) and the uncharged negative ions SO₂⁻ may penetrate into the polar region of the membrane and thus allow a fast reaction with the excited fullerene. The concentration of SO₂⁻ will only

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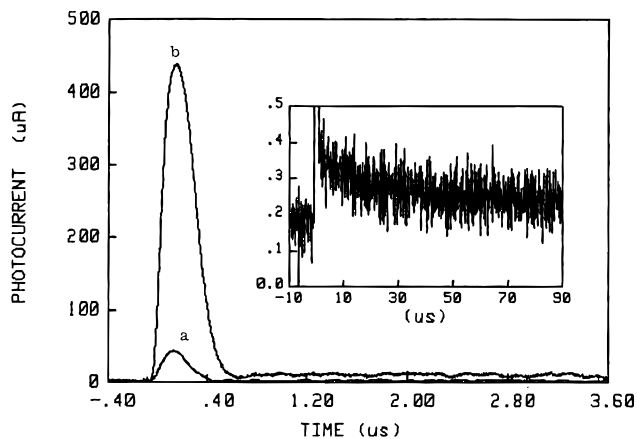


Figure 2. Photocurrent trace observed from planar lipid bilayers containing 0.6 mM C_{70} in the lipid solution. (a) Sodium dithionite (100 mM) is added to one aqueous compartment. The positive photocurrent is electron flow from the dithionite side. (b) Ferricyanide (10 mM) is added to the trans side of (a). An expanded scale of the slow component in (b) by filtering to 1 MHz is shown in the inset on a slower time scale. The excitation wavelength is 532 nm (100 μ J incident on the bilayer, 1 Hz). Each trace is an average of 16 pulses. A similar signal is obtained with a membrane containing C_{60} .

increase as the square root of $S_2O_4^{2-}$ concentration and may even saturate at the high concentrations of $S_2O_4^{2-}$ because of many side reactions of these species, e.g. tetrathionate formation.

(2) Photocurrent Measurements. Photocurrent measurements show more evidence of fast charge transport, although with lesser time resolution. In the dithionite| C_{70} | system without acceptor, the photocurrent shows a fast rise and decay with an instrument limited response time of <100 ns (Figure 2a). The negative current flows toward the acceptor compartment. On adding 5 mM ferricyanide as trans acceptor, dithionite| C_{70} |ferricyanide, the amplitude of this fast current is increased by a factor of 11 to 15 (Figure 2b), similar to the 15-fold increase of the photovoltage amplitude by the trans acceptor. In addition, a small component ($\sim 5\%$) of 6 μ s half-life appears (Figure 2, inset, and Table 1). Since this slow component of photocurrent only occurs after the addition of trans acceptor, it can be related to either the acceptor interfacial reaction or the transmembrane current. The latter implies that the charge transport across the bilayer is slower than the reaction at the acceptor interface, so that it must be independent of the concentration or kind of acceptor. Since the amplitude of the slow photocurrent component is very small compared to the fast one, the signal-to-noise ratio of the former was improved by filtering to 1 MHz. The results of varying the acceptor concentration are shown in Figure 3. The y-axis represents the decay rate constant, k . The finite rate constant at zero ferricyanide concentration is caused by the glucose oxidase used to remove oxygen. It has been shown to be a weak acceptor in the fullerene system.^{11,12} The rate constant increases linearly with ferricyanide concentration, and abruptly saturates beyond 3 mM. The function is much sharper than the usual hyperbolic saturation of a linear system and indicates changing gradients and fluxes as the acceptor concentration increases. An ohmic, not electronic, conductance is observed in the dark, and polarization currents can be detected on removing applied voltages. A similar saturation is seen for the amplitude of this slow component of photocurrent, but the amplitude of the fast component of the photocurrent whose rise time is instrument limited remains constant at all the ferricyanide concentrations. Since the current amplitude varies between individual membranes, the ratio of signal to noise is poorer than that of the kinetics shown in Figure 3. The saturated rate, with a half-time of 6 μ s, thus represents a membrane charge transit

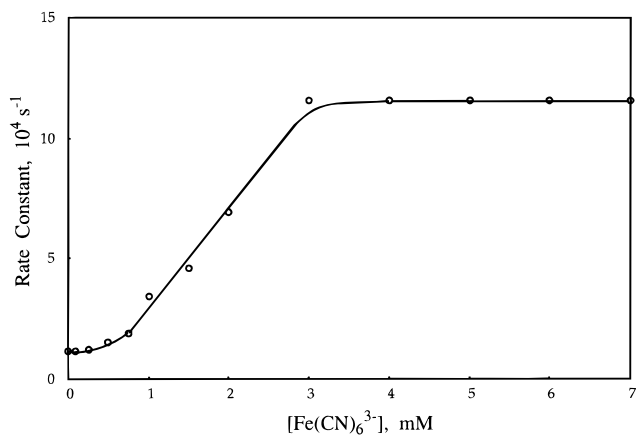


Figure 3. The decay rate constant of the slow photocurrent component is plotted versus the concentration of the acceptor ferricyanide. The opposite compartment contains 100 mM dithionite and the lipid membrane forming solution contains 0.5 mM C_{70} .

time. About all (ferricyanide) to $1/4$ (methyl viologen) of the 200-ns component can be accounted for by the C dV/dt response, with the membrane capacity $C = 4$ nF, from the interfacial charge transfer. Some of this response is a fast charge transit current (see below).

In the symmetric system with 100 mM dithionite present on both sides of the bilayer, 100 mM dithionite| C_{70} |100 mM dithionite, a photocurrent can also be observed when a voltage gradient is imposed. The photocurrent again is composed of a fast component with the system response time and a slow component of 7 μ s lifetime (Table 1). This time matches very well that of the dithionite| C_{70} |ferricyanide system and provides strong support that it is a charge transit time. Both fast and slow components of currents are linear in imposed voltage, but there is an offset in the current between negative and positive voltage. This offset can be explained by the polarization of the bilayer membrane. The life time of the slow photocurrent component was measured with voltage gradients from -80 to $+80$ mV for the symmetric system. The decay time, 7 μ s, does not change with the voltage gradient. If the transit time were determined by the movement of the fullerene anion, an increase with decreasing electric field would be expected. Given the short time, the conduction is more likely to be electronic.¹³ In that case the rate-determining step may be the rearrangement of the fullerene aggregates (see below) in setting up the electron hopping that constitutes the overall transit time.

The steady state photocurrent was measured using both a 532-nm cw laser (3 W/cm²) and a xenon arc lamp with interference filter (540 ± 6 nm, 10 mW/cm²). The observed photocurrent for the 100 mM dithionite|0.6 mM C_{70} |5 mM ferricyanide system is 20 and 0.13 nA, respectively (Table 1). At a laser power close to 10 W/cm², a current at 60 nA was observed, still showing no evidence of saturation. The estimated quantum yield of transmembrane photocurrent is 0.06 ± 0.02 assuming that the C_{70} -to-lipid ratio in the decane-toluene solution remains unchanged on forming the bilayer and using known values of absorption cross section of C_{70} . This result is similar to our previous finding of 0.04 for the 100 mM ascorbate|0.4 mM C_{70} |0.3 mM AQS system.¹²

(3) Transit Times and Fullerene Gradients. The nanosecond charge transit time is proof of electronic conduction. However, in addition to extremely fast and thus electronic charge conduction, one must consider the possibility that the added

acceptor has changed the distribution of fullerene in the bilayer. The dithionite is a strong enough donor ($E^0_{\text{SO}_3^{2-}/\text{S}_2\text{O}_4^{2-}} = -1.36 \text{ V}_{\text{SCE}}^{17}$) to reduce C_{70} molecules ($E^0_{\text{C}_{70}/\text{C}_{70}^{\bullet-}} = -0.43 \text{ V}_{\text{SCE}}^{7,18}$) in the dark, let alone reduce the triplet state ($E^0_{\text{C}_{70}/\text{C}_{70}^{\bullet-}} = +1.14 \text{ V}_{\text{SCE}}^{12,19}$) in the light. This was verified by the observation of dark current in the steady state measurements, which was negligible in the absence of acceptors (Table 1). The small photocurrents seen in their absence are caused by the glucose oxidase deoxygenation system which acts as a weak acceptor. In the absence of acceptor, the concentration ratio of fullerene and its anion will be about the same at both interfaces. The addition of trans acceptor causes a dark current and a possible gradient of the fullerene anion across the bilayer with a lower value at the acceptor interface and a complementary gradient of the neutral fullerene. This may increase the photoactive neutral fullerene at the donor interface and cause increase in the interfacial and trans membrane charge transfer on pulsed illumination. Because of the large difference in redox potentials, a large fraction of the fullerene may be reduced in the dark, with a correspondingly large change in the relative amount of reactive fullerene when the acceptor is added trans. An analysis with steady state kinetics for the dark reactions shows that the concentration of neutral fullerene at the donor interface will increase with high concentrations of acceptor for many values of rate constants.

There are two strong arguments against this view that a change in the interfacial concentration of C_{70} is responsible for the large increase in charge transfer on adding a trans acceptor. One is that the charge transferred as measured by $I_{t/2}$ of both fast and slow components is independent of ferricyanide concentration. Thus it represents a constant amount of photo-generated charge at the donor interface which is possible only if the C_{70} concentration is relatively constant. The second argument concerns the similarity between the response with the acceptors ferricyanide and methyl viologen. These compounds have standard redox potentials of +0.16 and -0.64 V vs SCE. Thus one would expect the effect of methyl viologen on C_{70}^- to be far less than that of the oxidant ferricyanide. Although the photovoltage is a factor of 5 less, the photocurrents are essentially the same (Table 1). A further argument is the similarity of the photocurrents in the symmetrical system with a polarizing voltage (40 mV) to that observed in the asymmetric ferricyanide system (32 mV total, Table 1), even though the redox properties of the two interfaces can hardly be more different. We conclude that the majority of the fast current component is a trans-membrane current with transit time < 100 ns and likely < 15 ns. Note that a polarization effect of this kind may be seen in the symmetric system in continuous light (Table 1) where the ratio of currents in the light to that in the dark is smaller than that in the asymmetric systems.

(4) Evidence for Active Fullerene Aggregates. Experimental evidence for fullerene aggregates comes from photovoltage measurements at different C_{70} concentrations in the lipid solution. Earlier measurements showed an "S" shaped response of photovoltage in the 30 mM ascorbate| C_{70} |0.2 mM AQS $^{2-}$ system, with increasing C_{70} concentration in the lipid bilayer solution.¹² However, the variation between necessarily different membranes with differing C_{70} concentration prevented drawing a strong conclusion. If the ratio of the photovoltage of the fast

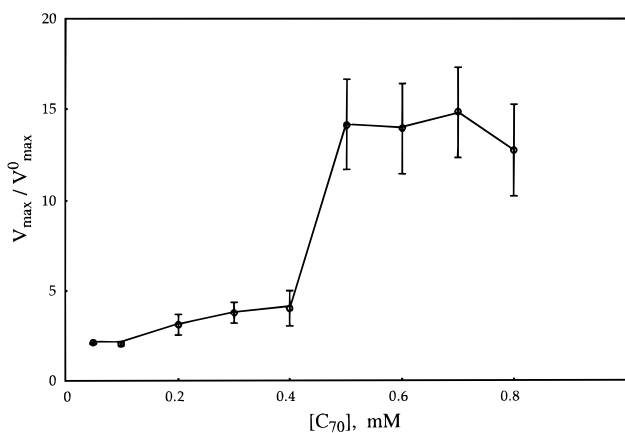


Figure 4. The ratio of photovoltage of the 100 mM dithionite| C_{70} |5 mM ferricyanide system (V_{max}) to the 100 mM dithionite| C_{70} system (V_{max}^0) in the same membrane is plotted versus the concentration of C_{70} in the lipid-forming solution. The data error at low C_{70} concentration is very small. The relatively large error at high C_{70} concentration may reflect variation in the distribution of size and number of C_{70} aggregates. The excitation is 532 nm (100 μJ , 1 Hz).

component in the dithionite| C_{70} |ferricyanide system to that of the dithionite| C_{70} system in the same membrane is plotted vs C_{70} concentration in the lipid solution, this normalization reduces the variation (Figure 4). At low C_{70} concentration, 0.05 to 0.1 mM, the photovoltage in the presence of trans acceptor ferricyanide is twice that in the absence of acceptor. The photovoltage ratio increases gradually from 0.2 mM C_{70} ($V_{\text{D-A}}/V_{\text{D}} = 3$) to 0.4 mM C_{70} ($V_{\text{D-A}}/V_{\text{D}} = 4$) and jumps to 15 ± 3 at ≥ 0.5 mM C_{70} in the lipid solution. The nonlinearity suggests that fullerene aggregates become more fully connected in the bilayer membrane when the C_{70} concentration in the lipid bilayer is high enough, i.e. > 0.5 mM. These aggregates would be very efficient in transporting the charge from donor interface to acceptor interface by electron hopping between the molecules in the aggregates.

Spectral evidence was found by Bensasson et al.²⁰ for aggregates of C_{70} in liposomes and by Sun and Bunker²¹ for aggregates of C_{70} in acetonitrile-toluene mixtures. In the lipid bilayer, C_{70} can be thought of as dissolved in a fatty acid ester. At both interfaces, there is water diffusion into the bilayer to some extent. Ethyl acetate-water mixtures can be utilized to mimic the C_{70} environment in the lipid bilayer. At a C_{70} concentration of 20 μM , the reddish orange solution in ethyl acetate (as in toluene) dramatically changes to pinkish purple when 1% water is added. The absorption spectrum of this mixture is obviously different from that in pure ethyl acetate and the two spectra are compared in parts a and b of Figure 5, respectively. Strong evidence for the photoactivity of the fullerene aggregates in the lipid bilayer is obtained from the measurement of the steady-state photocurrent as a function of excitation wavelength. The normalized photocurrents of the 100 mM dithionite| C_{70} |5 mM ferricyanide system are plotted vs the illumination wavelength for the same photon flux in Figure 5 (shown by +). They are compared to the absorption spectrum of C_{70} in the ethyl acetate (Figure 5a) and in the ethyl acetate-water mixtures (Figure 5b). It can be seen that the photocurrent plot is closer to the absorption spectrum of C_{70} in the ethyl acetate-water mixture, where the spectral changes suggest that

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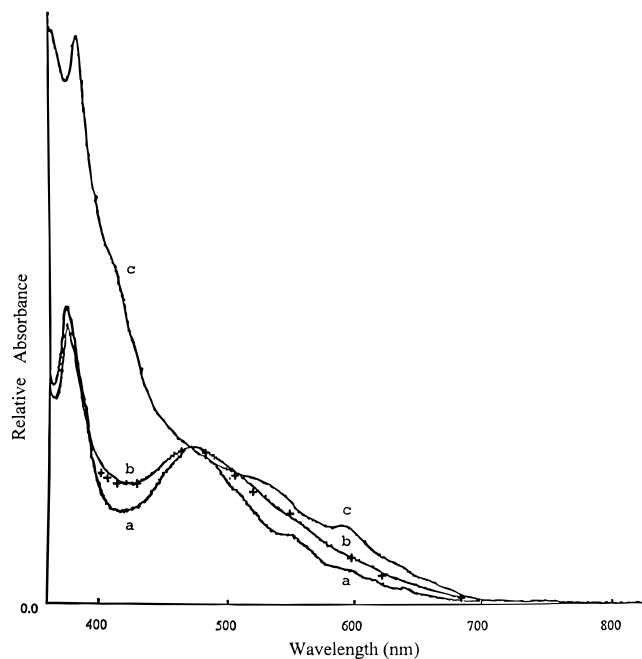


Figure 5. The steady state photocurrent normalized at 470 nm of the 100 mM dithionite| C_{70} |5 mM ferricyanide system is plotted as a function of excitation wavelength (+) for equivalent photon flux. The output of a 150-W xenon arc lamp with interference and blocking filters is used as the light source: (a) the absorption spectrum of C_{70} in ethyl acetate, (b) the absorption spectrum of C_{70} in ethyl acetate–water (1%) mixtures, and (c) the absorption spectrum of C_{70}^- (Gorum, private communication and ref 23), all normalized at 470 nm.

fullerene aggregates were formed. This result strongly supports the existence of C_{70} aggregate in the lipid membrane in the 100 mM dithionite|0.6 mM C_{70} |5 mM ferricyanide system and also shows that the aggregate is photoactive. The data shown in Figure 4 support the view that these aggregates transport charge across the lipid bilayer not only very fast but also very efficiently. The absorption spectrum of C_{70}^- is also shown in Figure 5c²³ and the C_{70}^{2-} anion is even more highly absorbent in the 400-nm region than that of C_{70}^- (not shown). Therefore, these anions clearly contribute little to the photoactivity.

Conclusion

There are two kinds of charge transfer mechanism across a lipid bilayer: electronic and diffusive ionic conductances. As argued before,¹³ an observed charge transit time of $<10 \mu\text{s}$ would be evidence for electronic conduction and a much shorter time would constitute proof of this mechanism. This argument is based on the known mobility of the similarly sized tetraphenyl borate ion (0.8 nm vs 1.0 nm for C_{70}) in water ($2 \times 10^{10} \text{ nm}^2$

$\text{s}^{-1} \text{ V}^{-1}$), an internal membrane thickness of 5 nm, a viscosity of 10 P, and a trans membrane voltage of 10 mV, resulting in a minimum transit time of $30 \mu\text{s}$. The electrostatic (Born charging) energy of the ion in the hydrocarbon core of the membrane will add a considerable barrier to this crossing time. Our measurements show that the bilayer internal transit time of similar sized tetraphenyl borate ions is $\sim 1 \text{ ms}$.²² The photovoltage and photocurrent measurements discussed above have shown strong evidence that the transit times of charge transport across the lipid bilayer are $<100 \text{ ns}$ and $\sim 6 \mu\text{s}$. These short transit times indicate that the dominant charge transport is an electronic conductance. Attempts to independently verify electronic versus diffusive conductance by using a lipid below its gel transition temperature were frustrated by membrane instability (Hwang and Mauzerall, unpublished).

The interfacial electron transfer from donor to excited C_{70} can occur in less than 15 ns with a sufficiently reactive donor, dithionite (Figure 1). Both the photovoltage and photocurrent increase markedly when an electron acceptor is added to the trans membrane side (Figure 2). Thus efficient charge transfer is occurring completely across the lipid membrane and the transit time is $<100 \text{ ns}$ and could be $<15 \text{ ns}$, fully justifying an electronic as opposed to a diffusive mechanism of conductance.

The linear relationship between the rate constant of the slow component of the photocurrent and acceptor concentration followed by its saturation at high concentration indicate that this saturated rate is a charge transfer rate across the membrane (Figure 3). Thus this charge transit time is $6 \mu\text{s}$. The photocurrent of the symmetric system, dithionite| C_{70} |dithionite, driven by imposed voltage, shows the same transmembrane transit times further supporting this conclusion. The independence of this time with applied voltage indicates a possible rearrangement time in the active C_{70} aggregates. Possibly this fraction of the aggregates is smaller than the fast fraction and requires movement or another C_{70} to cross the bilayer.

The strongly nonlinear dependence of the ratio of photovoltage in the presence of trans acceptor to that in its absence suggests that aggregates of C_{70} are responsible for the efficient transport of charge across the membrane (Figure 4). At a critical concentration the aggregates form a conductive path across the bilayer. The variation of steady-state photocurrent with the excitation wavelength shows an action spectrum plot closer to the absorption spectrum of C_{70} aggregates in the ethyl acetate– H_2O mixture than the absorption spectrum of C_{70} molecule in ethyl acetate or that of fullerene anion (Figure 5). This provides strong evidence of the existence of photoactive C_{70} aggregates in the lipid bilayer. Fullerene aggregates can thus rapidly and efficiently transport charge across the lipid bilayer.

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