Vectorial Photoinduced Electron Transfer between Phospholipid Membrane-Bound Donors and Acceptors

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Abstract: Photoinduced electron transfer (PET) from the hydrophobic triphenylbenzylborate anion to the water-soluble tricationic *N,N'*-bis[3-(trimethylammonio)propyl]thiadicarbocyanine was investigated in water and in phosphatidylcholine membrane systems. The borate (donor) and dye (acceptor) form a strong complex in water, leading to inefficient PET. In the presence of liposomes, the hydrophobic borate anion is bound deep within a bilayer. The resulting negative surface charge of the liposomes leads to electrostatic binding of the cationic dye within the head group region of the membrane. This faciliates PET to the photoexcited dye, as observed in photobleaching and fluorescence quenching experiments. The important feature of the system is the simultaneous binding of both donor and acceptor at relatively well-defined but nonoverlapping binding sites within the membrane, leading to vectorial PET from the hydrophobic interior of the membrane to the hydrophilic surface upon red light irradiation. Moreover, the PET efficiency is dependent on the physical state of the bilayer, which indicates that the borate moves closer to the dye at temperatures below the lipid phase transition temperature (T_m), a probable consequence of the tighter packing of the lipids in the solid-analogous phase.

Introduction

The photoinduced transfer of an electron across a phospholipid membrane in photosynthesis is one of nature's most remarkable feats. The pathway followed by the electron is extremely intricate, consisting of a series of short hops between various redox components embedded within a transmembrane protein.[1,2] The result of this process is the separation of charge by ca. 40–50 Å, precluding back electron transfer. The energy stored in the charge-separated state is then used to drive subsequent chemical processes. The ability to mimic these features of photosynthesis is of immense practical importance for applications such as solar energy conversion. However, because of the sheer structural complexity of the reaction center, simplified systems employing liposomes (vesicles)[3] have been used to achieve transmembrane electron transfer.[4-6] In these systems, the redox species are either bound to the surface of the membrane or within its hydrophobic interior. Transmembrane electron transfer thus results from a series of short, discrete hops (truly mimicking reaction centers) and/or electron transport via a lipophilic carrier. Of course a major difference between these model systems and photosynthetic reaction centers is that in the former the medium is a bilayer membrane whereas in the latter the medium is a protein.

Considerably less attention has been given to studying photoinduced electron transfer (PET) reactions occurring either completely within the bilayer or across a single interface of a phospholipid membrane, despite the fact that virtually any strategy devised to achieve efficient transmembrane PET will require these types of reactions to occur. Intrabilayer electron transfers are especially problematic because of the difficulty involved in establishing the locations of both the donor and the acceptor in the membrane. This situation is simplified to some extent by following transfer across the membrane interface either from a donor in the bilayer to an acceptor in the aqueous phase (electron ejection) or vice versa (electron injection). In particular, Tsuchida observed a strong inverse distance dependence on the efficiency of photoinduced electron ejection from amphiphilic porphyrins bound at different depths in the bilayer to acceptors in the aqueous medium.[7]

The phospholipid bilayer is a medium unto itself, with its own volume, polarity, and fluidity. The latter property can be readily modified by incorporation of cholesterol into the membrane by variation of the sample temperature with respect to the phase transition of the membrane or by polymerization of the bilayer.[8,9] Hiromitsu and Kevan have measured photoionization yields of dipalmitoylphosphatidylcholine (DPPC) membrane-bound chlorophyll *a* (Chla) in the presence of electron scavengers in the aqueous phase with variable amounts of cholesterol in the membrane.[10] The yields decreased with increasing cholesterol concentration in apparent contradiction with the results obtained by Ford and Tollin, who observed that the extent of electron-transfer quenching of Chla triplets in egg phosphatidylcholine liposomes increased with increasing cholesterol concentration.[11] Hiromitsu and Kevan suggested that the conflicting results might be due to different cholesterol–lipid interactions for the two sets of experiments, but other variables include the techniques and types of samples used (steady-state ESR on frozen liposome suspensions at 77 K for Hiromitsu and Kevan, transient absorbance on room-temperature liposome suspensions for Ford and Tollin). The broader point to be made is that, while inclusion of cholesterol in the membrane more closely mimics biological membranes, it also introduces significant structural heterogeneity into the bilayer which makes data analysis difficult.

The effect of membrane fluidity on PET reactions may also be examined by thermally controlling the fluidity. Ottolenghi and co-workers followed this strategy in their study of PET from an amine donor to excited pyrene confined within DPPC liposomes.[12] The electron-transfer reaction was more efficient at temperatures above the phase transition of the membrane, where the bilayer was fluid, than at lower temperatures, where the bilayer was in the solidlike gel state. Similar results were obtained by Kano and co-workers.[13] However, in neither case were the locations of the donor and acceptor within the membrane clearly established.

In this report, we present studies of PET reactions confined to a phospholipid membrane in which the donor and acceptor occupy reasonably well-defined binding sites. The donor, triphenylbenzylborate anion, forms extremely strong aqueous complexes

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with various cationic dyes, leading to relatively inefficient PET between them. Liposomes, however, enhance the efficiency of the electron-transfer process by preventing complex formation.[14] Many approaches have been developed which employ organized assemblies to achieve enhanced PET efficiency with respect to a homogeneous aqueous medium.[15] For example, Willner and associates have used charged colloids[16] and micelles[17] to disrupt aqueous charge-transfer complexes. The aqueous complexes exhibited inefficient PET due to rapid back electron transfer. However, more efficient PET occurred when one component was bound to the charged interface while the other was repelled into the aqueous medium, the back electron transfer being retarded due to the much larger distance between the redox products. The current study is unique in two important ways: (1) The liposomes bind both redox components simultaneously. The donor used in this study belongs to a class of molecules known as hydrophobic ions, characterized by their ability to partition overwhelmingly from aqueous media into neutral or anionic phospholipid membranes.[18-20] The cationic acceptor is then bound to the liposomes by electrostatic attraction. Although donor and acceptor are both bound within the membrane, they are bound at different depths owing to the polarity[21] and fluidity[22] gradients inherent in the bilayer. This permits efficient electron transfer to occur between the two while the bilayer interface inhibits complex formation. (2) Although anionic liposomes work quite well at disrupting dye–borate complex formation and enhancing net PET between them, the liposomes need not be charged. Binding of hydrophobic borate anions to neutral liposomes imparts sufficient negative charge to the membranes to induce binding of cationic water-soluble dyes. Moreover, the binding site of the donor (but not the acceptor) appears to be sensitive to the physical state of the membrane, which is simply a function of temperature. Control over the efficiency of the PET reaction is thus obtained from the resulting variable distance between donor and acceptor.

Experimental Section

Materials. *N,N'*-Bis[3-(trimethylammonio)propyl]thiadicarbocyanine tribromide (Cy^{3+}) was purchased from Molecular Probes (Eugene, OR) and used as received. Rhodamine 6-G (R6G) was purchased from Eastman Kodak Co. (Rochester, NY) and used as received. 5,10,15,20-Tetrakis[4-(trimethylammonio)phenyl]-21*H*,23*H*-porphine tetra-*p*-tosylate salt (TAPP), sodium tetraphenylborate (Ph_4B^-), *n*-octanol (HPLC grade), ethanol, *n*-propanol, and *n*-butanol (all spectral grade) were purchased from Aldrich Chemical Company (Milwaukee, WI) and used as received. *n*-Pentanol (99%) was purchased from Aldrich and purified by fractional distillation from calcium hydride immediately before use. Methanol and acetonitrile (J. T. Baker Inc., Phillipsburg, NJ; Photrex grade) were used as received. L-α-Dipalmitoylphosphatidylcholine (DPPC), L-α-dioleoylphosphatidylcholine (DOPC), and L-α-dioleoylphosphatidic acid (DOPA) were purchased from Avanti Polar Lipids (Pelham, AL) and used as received. Purity was checked by thin-layer chromatography (65:25:4 $CHCl_3$/MeOH/H_2O eluent). Lipids were stored as chloroform solutions at 5 °C.

The tetramethylammonium salts of triphenylbenzylborate (Ph_3BnB^-) and triphenyl-*n*-butylborate were synthesized according to published procedures.[23,24] The white crystalline solids gave satisfactory ^1H NMR spectra and melting (decomposition) points.

Spectroscopy of Aqueous Dye–Borate Complexes. UV–vis absorption spectra were acquired on a Varian DMS-200 (Varian Techtron Pty. Limited, Victoria, Australia) double-beam instrument. The absorption spectrum of a 3.85 μM aqueous Cy^{3+} solution (1.3 mL) was recorded before and after the addition of successive 1.0-μL aliquots of a 1.0 mM borate (Ph_3BnB^- and Ph_4B^-) stock solution (CH_3CN).

Fluorescence spectra were acquired on a Spex Fluorolog II spectrofluorometer (Spex Industries, Inc., Edison, NJ). Spectra of aqueous Cy^{3+} and Cy^{3+}–borate complexes were obtained with excitation at 671 nm, the isosbestic point observed in the absorption spectra during complex formation.

The stoichiometry of the Cy^{3+}–Ph_3BnB^- complex was determined using Job's method.[25] Stock solutions employed were 1.0 mM Cy^{3+} in water and 1.0 mM Ph_3BnB^- in CH_3CN. The total volume of these solutions added to 1.3 mL of water was held constant at 12.0 μL. Initially, 12.0 μL of Cy^{3+} and 0 μL of Ph_3BnB^- were added and the absorption spectrum recorded. The procedure was repeated for 10:2, 8:4, 6:6, 4:8, 2:10, and 0:12 (μL of Cy^{3+}:μL of Ph_3BnB^-) mixtures of the two components. The optical density of the solution at 700 nm was used to assay for complex formation as the free dye has only a minor absorbance at this wavelength. The data was corrected for residual free dye absorbance.

Liposome Preparation. An appropriate amount of lipid stock solution was added to a 10-mL pear-shaped flask. The solvent was evaporated by passing a stream of argon gas over the sample, leaving a film of lipid on the wall of the flask. Residual solvent was removed by drying under vacuum for at least 8 h. The lipid film was hydrated with 4.0 mL of buffer (10 mM imidazole, pH 7.0), warmed above the lipid phase transition (for DPPC), and vortexed. The sample was then subjected to 10 freeze–thaw cycles, accomplished by immersion for 4 min in a dry ice/isopropyl alcohol bath followed by 10 min in a water bath. For DPPC preparations, the sample was placed in a 60 °C bath for 1 min prior to each freeze–thaw cycle. Liposomes were formed by extrusion 10 times through two 0.1-μm polycarbonate filters (Nuclepore Co., Pleasanton, CA) at a pressure of 250 psi.[26] The extruder (Lipex Biomembranes, Inc., Vancouver, B.C.) was warmed to 50 °C for preparation of DPPC liposomes.

Binding of Cy^{3+} to DOPC/DOPA (9:1) and DOPC Liposomes. DOPC/DOPA (9:1) liposomes were prepared as described above. A total of 4.0 μL of a 1.0 mM aqueous Cy^{3+} solution was added to 1.3 mL of the liposome suspension (0.5 mg of lipid/1 mL of buffer). The UV–vis absorption spectra were recorded with a cuvette containing only liposomes in the reference beam. Then 4.0 μL of a 2.0 mM Ph_3BnB^- stock solution (CH_3CN, 2.0 molar equiv, relative to Cy^{3+}) was added and the absorption spectrum recorded. (Note: In all experiments, the volume of added organic solvent was less than 1% of the total volume.) The sample was then irradiated at room temperature as described below for a total of 10 min with absorption spectra recorded at 2-min intervals. The entire procedure was repeated using pure DOPC liposomes. All amounts of reagents were the same as in the DOPC/DOPA experiment, except that 6.0 μL of a 2.0 mM Ph_3BnB^- stock solution (CH_3CN, 3.0 molar equiv relative to Cy^{3+}) had to be used, as explained in the text.

Differential Scanning Calorimetry. DPPC (8 mg) was dried from a stock chloroform solution onto the wall of a 10-mL pear-shaped flask. After drying in vacuo overnight, the sample was hydrated with 4.0 mL of buffer (10 mM imidazole, pH 7.0), warmed to 50 °C, and vortexed. The sample was then subjected to 10 freeze–thaw cycles. To the sample were then added 5.2 μL of 50.0 mM Ph_4B^- stock (4.8 mol %), 3.3 μL of 10.0 mM Ph_3BnB^- stock (0.6 mol %), and 18.0 μL of 1.0 mM Cy^{3+} stock (0.33 mol %). DSC thermograms were recorded using a Microcal MC-2 calorimeter (Microcal, Inc., Northampton, MA). Four consecutive scans were acquired from 20 to 60 °C at a heating rate of 10 °C/h.

Steady-State Irradiations. Irradiations were performed using red light obtained from the filtered output of a 200-W Hg(Xe) arc lamp (Oriel Corp., Stratford, CT). CS2-62 (Corning Glass Works, λ > 580 nm) and neutral density (6% transmitting) filters were used. The samples (1.3 mL) were irradiated in a quartz cuvette at a distance of 22.4 cm from the lamp. The sample temperature was controlled by using a thermostated cell holder connected to a water circulator. Lamp flux (ca. 10^{16} photons/s) was measured using a thermopile detector having a surface area of 0.04 cm^2.

Photoinduced Electron Transfer in DPPC Liposomes: Photobleaching. DPPC liposomes (0.5 mg/mL) were prepared as described above. The liposomes (1.3 mL in a quartz cuvette) were warmed to 50 °C before addition of dye and borates. Then, 4.3 μL of 10.0 mM Ph_4B^- stock (4.8 mol %), 2.7 μL of 2.0 mM Ph_3BnB^- stock (0.6 mol %), and 1.8 μL of 1.0 mM Cy^{3+} stock (0.2 mol %) were added. The tetraphenylborate was included as an inert hydrophobic anion to bind the dye to the surface of

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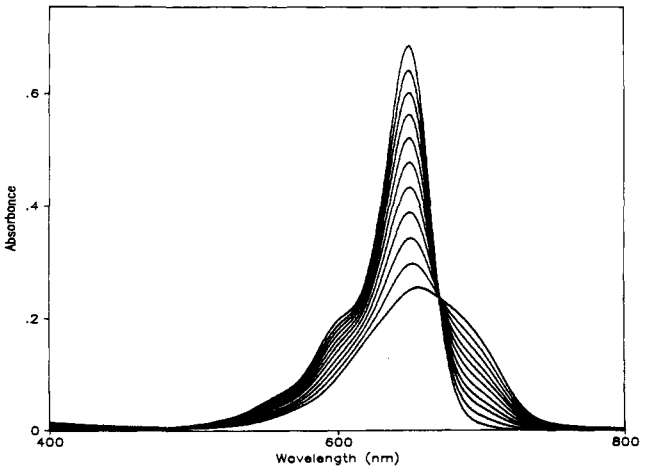


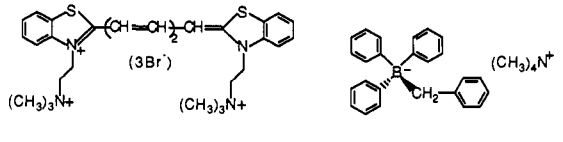
Figure 1. Absorption spectra of 3.85 μM aqueous Cy^{3+} titrated with successive additions of 0.2 molar equiv of Ph_3BnB^-.

the liposome. The sample was then incubated at the desired temperature for 15 min in the dark prior to irradiation. During this time, no change was observed in the absorption spectrum of the dye. Irradiation was then performed as described above. Absorption spectra were recorded at different times up to 10 min total irradiation. The sample cell holder in the instrument was kept at the same temperature as the irradiation cell holder to prevent temperature change during the acquisition of the spectrum. The percent bleaching of the dye was calculated from the loss in maximum optical density. The time required to reach 50% bleaching was determined graphically and plotted versus temperature.

Fluorescence Quenching. Sample preparation was as described above for the photobleaching experiments except for the total amounts of reagents used. For these experiments, 3.0 mL of liposome suspension (0.1 mg/mL) was used in a fluorescence cuvette, and 2.0 μL of 10.0 mM Ph_4B^- stock (4.8 mol %) and 8.2 μL of 0.1 mM Cy^{3+} stock (0.2 mol %) were added. Variable amounts of Ph_3BnB^- were also added. In this way, although the concentration of liposomes is lower than in the photobleaching experiments, the number of dye and borate molecules per liposome remains consistent. The cell holder in the fluorometer was connected to a water circulator to afford temperature control. After 15 min of incubation at the desired temperature, the emission spectrum (excitation at 670 nm) was acquired. A fresh sample was used for each measurement.

Results

Aqueous Dye–Borate Complexes. The water-soluble, tricationic dicarbocyanine dye I (Cy^{3+}) selected for the study exhibits intense absorption in the red (λ_{max} = 650 nm in water, ϵ_{max} = 130 000). Figure 1 shows the results of titration of Cy^{3+} with triphenylbenzylborate II (Ph_3BnB^-). The broad, red-shifted absorbance and decreased extinction coefficient indicate complex formation. Noteworthy is the observation that the complex appears to be quite strong: each successive curve corresponds to the addition of just 0.2 molar equiv of borate. Nearly identical spectral perturbations are observed for the titration of I with Ph_4B^-.



I (Cy^{3+}) II (Ph_3BnB^-)

Job's method[25] was used to determine the complex stoichiometry as shown in Figure 2. The maximum optical density due to the complex occurs at χ = 0.67, corresponding to a 1:2 (Cy^{3+}:Ph_3BnB^-) complex. This gives the complex an overall charge of +1. Also of significance is the extremely sharp peak observed in the plot, which is characteristic of a strongly associated complex. The binding constant for the complex cannot be determined by simple Benesi–Hildebrand[27] treatment of the data since the approximations implemented in that method do not apply to strong

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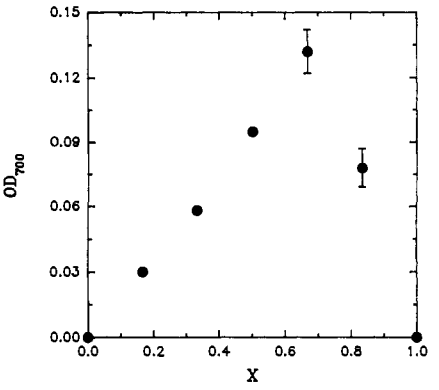

Figure 2. Job plot for formation of the aqueous Cy^{3+}–Ph_3BnB^- complex. Each data point and set of error bars represent the mean and standard deviations, respectively, for three separate measurements.

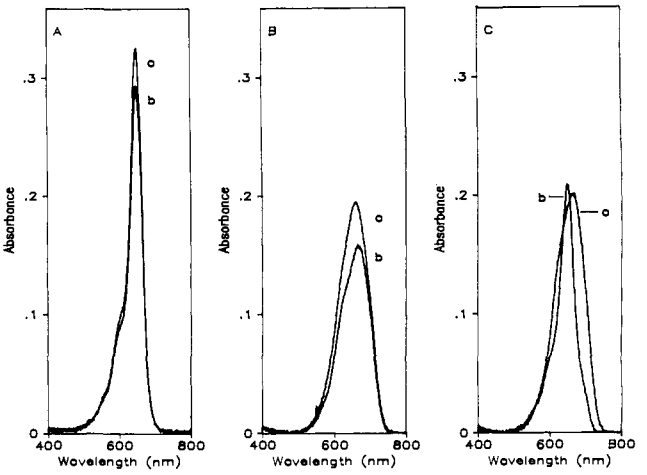

Figure 3. Absorption spectra of solutions of (A) 2.5 μM aqueous Cy^{3+}; (B) 3.1 μM aqueous Cy^{3+}–Ph_4B^- (1:2); and (C) 3.1 μM aqueous Cy^{3+}–Ph_3BnB^- (1:2) before (curve a) and after (curve b) 10 min of red light irradiation.

associations. However, the observations that the complex has 1:2 (Cy^{3+}:Ph_3BnB^-) stoichiometry and that the clean isosbestic point observed in Figure 1 is lost as soon as the ratio of Ph_3BnB^- to Cy^{3+} exceeds 2.0 imply that there is virtually no uncomplexed borate. Rather than being in equilibrium with the dissociated components, the aqueous complexes appear to be discrete, supramolecular species.

Ph_3BnB^- also forms aqueous complexes with other positively charged dyes such as a tetracationic porphyrin (TAPP) and monocationic rhodamine 6-G (R6G). Similar analysis of these complexes reveals stoichiometries of 1:3 (TAPP:Ph_3BnB^-) and 2:1 (R6G:Ph_3BnB^-) and sharp maxima in the Job plots. Both stoichiometries correspond to complexes with a net charge of +1.

The results of red light irradiation of Cy^{3+} alone and when complexed with either Ph_3BnB^- or Ph_4B^- are shown in Figure 3. (The experiments show good reproducibility; the spectra in Figure 3 are typical results.) The concentration of Cy^{3+} in the absence of borate was adjusted such that the total optical density in the region of irradiation ($\lambda > 580$ nm) was equivalent to that of the complexes. The dye alone (Figure 3A) and the Cy^{3+}–Ph_4B^- complex (Figure 3B) bleach slowly and uniformly over 10 min of irradiation. However, the Cy^{3+}–Ph_3BnB^- complex (Figure 3C) exhibits quite different behavior. Irradiation leads to drastic changes in the absorption spectrum, with the peak shifting from 665 back to 651 nm and becoming much more narrow. Apparently uncomplexed dye is released as the reaction proceeds. Equal loss of cyanine and borate (on a percentage basis) should lead to uniform bleaching, since the remaining dye would still be exposed to 2 equiv of borate, but this is clearly not observed. The extent of complexation after 10 min of irradiation can be determined using the titration data. The ratio OD_{650}:OD_{671} is equal

Table I. Percent Bleaching of Cy^{3+} in H_2O and DOPC/DOPA Membranes after 10 min of Red Light Irradiation

conditions	H_2O	DOPC/DOPA
Cy^{3+} Alone	6.8	3.5
Cy^{3+}/Ph_4B^-	15.5	1.6
Cy^{3+}/Ph_3BnB^-	15	74.1

Table II. Quenching of Cy^{3+} Fluorescence by Borates

borate	% quenching in H_2O[a]	% quenching in DOPC/DOPA
2 equiv of Ph_4B^-	70 (4)	−5[b]
2 equiv of Ph_3BnB^-	91 (1)	32

[a] Mean of six measurements; standard deviation in parentheses. [b] 5% enhancement of Cy^{3+} fluorescence upon addition of 2 equiv of Ph_4B^-.

to 1.6 in Figure 3C. On the basis of the titration data, this would correspond to a 1:1.4 ratio of Cy^{3+} to Ph_3BnB^-. If no borate remained, a spectrum corresponding to free Cy^{3+} and having OD_{650} = 0.37 would result, on the basis of the spectra in Figure 1. In the absence of borate and prior to irradiation, OD_{650} = 0.44. Thus, ca. 15% bleaching of Cy^{3+} and 41% loss of borate occurred in 10 min of irradiation. (These results suggest that either the reduced dye formed by PET from Ph_3BnB^- to excited Cy^{3+} is being oxidized back to Cy^{3+} or that the borate is consumed by some process in addition to direct oxidation by excited Cy^{3+} within the complex). Thus, both complexes are more photosensitive than the dye by itself, with the Ph_3BnB^- complex being the less stable of the two toward red light. The results for irradiation in water as well as anionic liposomes (vide infra) are summarized in Table I.

The fluorescence of aqueous Cy^{3+} is strongly quenched by complex formation with the borates. The emission spectra were acquired with excitation at the isosbestic point (671 nm) observed in the absorption spectra for titration of Cy^{3+} with either borate. Excitation at this wavelength guarantees the same number of excited species (free dye or complex) for each measurement. As seen in Table II, the fluorescence is quenched considerably more efficiently in the Ph_3BnB^- complex.

Experiments with Anionic Liposomes. Addition of 3.1 μM Cy^{3+} to DOPC/DOPA (9:1) liposomes (0.5 mg/mL) causes a significant bathochromic shift of the absorption spectrum of the dye (Figure 4) indicative of binding of the dye to the liposomes. These concentrations correspond to approximately 8×10^3 DOPA molecules per liposome (slightly more than half of which are accessible to the externally added Cy^{3+}) and 350–400 Cy^{3+} per liposome (assuming complete binding). The lack of change in the dye absorption profile upon binding indicates that the dye is bound as monomers. This is ascribed to the relatively low dye surface concentration (less than one Cy^{3+} for every hundred lipids) and the electrostatic repulsion between the highly charged dye molecules at the bilayer surface. In addition, aggregation of membrane-bound dyes is typically more favorable when the membrane is in the gel state.[28,29] The DOPC/DOPA liposomes used in this experiment were in the more fluid liquid crystalline state.

Information regarding the binding site of Cy^{3+} within the membrane can be ascertained by examining the solvatochromism of the dye. Figure 5 shows the dependence of the absorption maximum of Cy^{3+} on solvent dielectric constant for a series of primary alcohol solvents. The observed maxima when bound to the DOPC/DOPA liposomes (669 nm) suggests that the dye is bound within a region of fairly low dielectric constant (ϵ = 13–14), i.e., the dye penetrates significantly into the head group region of the bilayer.

Binding of Cy^{3+} to the anionic liposomes results in an 89% increase in the fluorescence yield relative to aqueous Cy^{3+}. Since

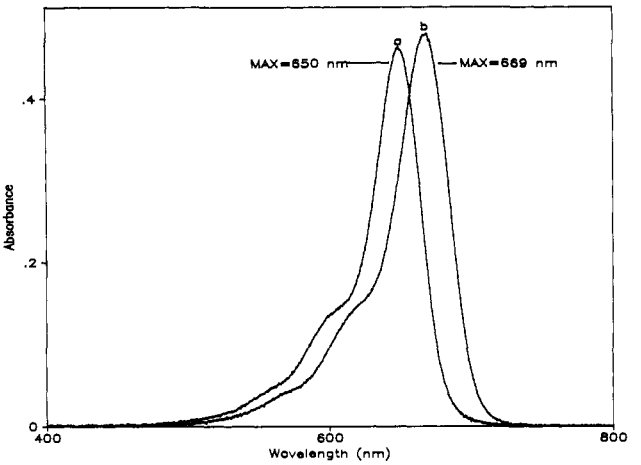

Figure 4. Absorption spectra of 3.1 μM Cy^{3+} in imidazole buffer alone (curve a) and with DOPC/DOPA liposomes (curve b). Approximately 350–400 Cy^{3+} bound per liposome.

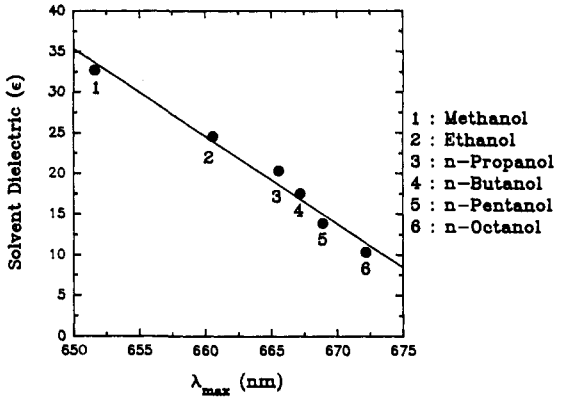

Figure 5. Plot of absorption maximum versus solvent dielectric constant for Cy^{3+} dissolved in a series of primary alcohols. Dielectric constants were taken from ref 48.

the predominant decay path for excited cyanines in solution is nonradiative decay facilitated by twisting about the methine bridge,[30-32] placing the dye in a more rigid environment (i.e., the membrane surface) should lead to enhanced fluorescence by restricting this twisting motion. Thus, fluorescence quantum yields for cyanines are known to increase significantly with increasing solvent viscosity.[33-36] Additionally, Kunitake and co-workers observed fluorescence enhancements for binding of anionic cyanines to cationic ammonium bilayer interfaces,[37] while Sukigara and associates found that incorporation of amphiphilic cyanines into bilayers gives similar results.[38]

Addition of 2 molar equiv of either Ph_3BnB^- or Ph_4B^- (relative to Cy^{3+}) to DOPC/DOPA liposomes (700–800 borates per liposome) with surface-associated Cy^{3+} has very little effect on the absorption spectrum, aside from a slight (<5%) decrease in the extinction coefficient. Formation of a dye–borate complex appears to be inhibited in the presence of anionic liposomes. The two borates exhibit very different quenching abilities in DOPC/DOPA membranes. As seen in Table II, 32% of membrane-bound Cy^{3+}

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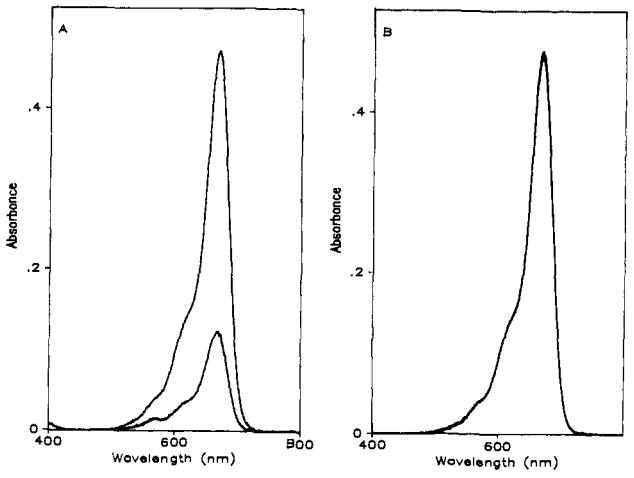


Figure 6. Effect of 10 min of red light irradiation on the absorption spectra of Cy^{3+} bound to DOPC/DOPA liposomes in the presence of 2 molar equiv of (A) Ph_3BnB^- and (B) Ph_4B^- (λ_{max} = 669 nm for both A and B). In each case, approximately 350–400 Cy^{3+} and 700–800 borates were bound per liposome.

fluorescence is quenched by 2 molar equiv of Ph_3BnB^-, whereas addition of Ph_4B^- slightly enhances the fluorescence. Ph_3BnB^- and Ph_4B^- also yield drastically different results for bleaching experiments. Irradiation of the membrane-bound Cy^{3+} after addition of Ph_3BnB^- leads to rapid bleaching of the dye (Figure 6A). If Ph_4B^- is present instead, the dye is extremely stable to irradiation (Figure 6B). In the absence of either borate, minimal bleaching of the dye occurs. The bleaching results are collected in Table I.

Experiments with Neutral Liposomes. Experiments identical to those described above for anionic DOPC/DOPA liposomes were performed on pure DOPC liposomes in a 10 mM phosphate buffer. In the absence of borate, Cy^{3+} does not interact with the electrically neutral liposomes. However, addition of Ph_3BnB^- induces binding of the dye to the liposomes. This is the result of partitioning of the negatively charged borate into the phospholipid bilayer, rendering the liposome anionic. The cationic dye is then electrostatically bound to the surface, as evidenced by spectral shift (not shown) identical to that observed in Figure 4. Irradiation again leads to rapid bleaching of Cy^{3+} as observed in Figure 6A, but with a progressive hypsochromic shift with increasing irradiation time. Since oxidation of Ph_3BnB^- results in destruction of the negative charge required to bind Cy^{3+}, irradiation causes the negative potential of the membrane to decrease. Unbleached dye begins to dislodge from the liposome surface, causing the hypsochromic shift. In addition, inclusion of 100 mM sodium chloride in the phosphate buffer effectively screens out the charge given to the liposomes by the borate, preventing binding of the cyanine to the liposomes.

Effect of Membrane Physical State on PET from Ph_3BnB^- to Cy^{3+}. DPPC bilayers exhibit a highly cooperative main phase transition (T_m) at 41.4 °C between a solidlike gel state at low temperatures and a more fluid liquid crystalline state at high temperatures. Thus, DPPC liposomes permit investigation of the influence of the membrane physical state on processes occurring within the bilayer over a fairly wide temperature range. (In these experiments and the fluorescence quenching experiments described below, an excess of Ph_4B^- was used to keep Cy^{3+} bound to the liposomes for the duration of the experiment. Control experiments showed that liposomal Cy^{3+} was not photobleached in the presence of Ph_4B^-. In all cases, the dye absorption maximum and profile were consistent throughout the irradiation, indicating that the dye remained bound to the liposomes during the experiment.) Photobleaching of Cy^{3+} by Ph_3BnB^- was studied in DPPC liposomes from 25 to 55 °C. The time required to reach 50% bleaching of the dye is plotted versus temperature in Figure 7, which shows that the bleaching is 3.5 times faster at temperatures below T_m than above. A sharp discontinuity in the plot is observed in the region of the phase transition, although it is somewhat below the

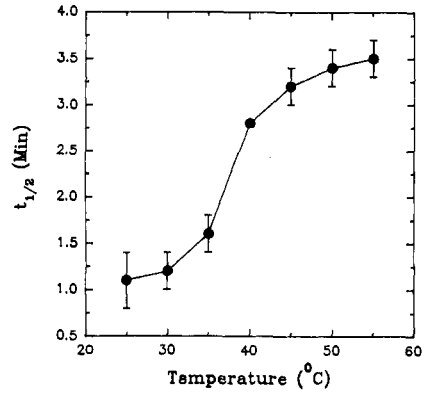


Figure 7. Effect of temperature on the half-life rate of photobleaching of 1.38 μM Cy^{3+} during red light irradiation in the presence of 4.15 μM Ph_3BnB^- in DPPC liposomes (0.5 mg/mL). Each data point and set of error bars represent the mean and standard deviations, respectively, for three separate measurements.

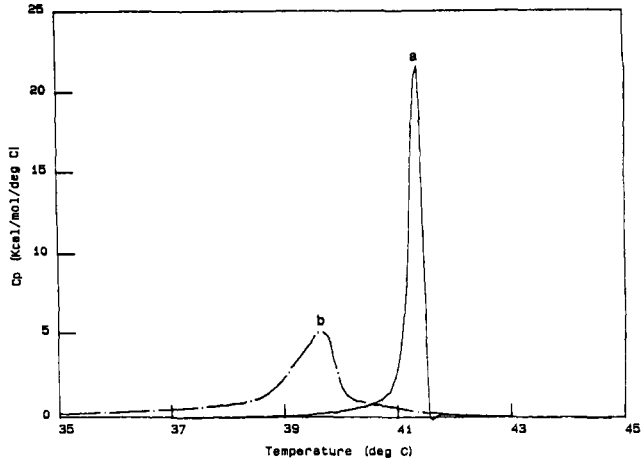


Figure 8. DSC thermograms for DPPC alone (curve a) and in the presence of Cy^{3+}, Ph_3BnB^-, and Ph_4B^- (curve b) in the relative amounts employed in the photobleaching and fluorescence quenching experiments.

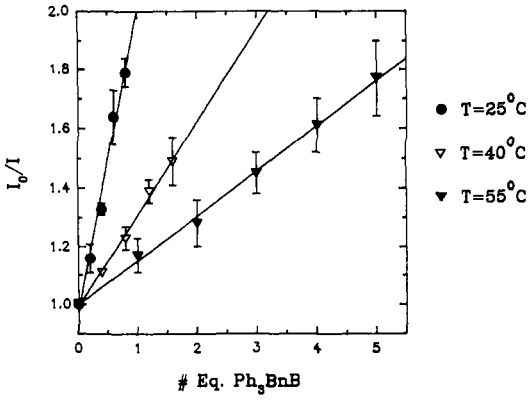
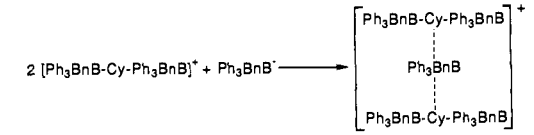


Figure 9. Effect of temperature on the fluorescence quenching of 0.27 μM Cy^{3+} by Ph_3BnB^- in DPPC liposomes (0.1 mg/mL). Each data point and set of error bars represent the mean and standard deviations, respectively, for three separate measurements.

41.4 °C value of T_m for DPPC. To examine whether the discontinuity observed in Figure 7 corresponds to the phase transition of the liposomes, differential scanning calorimetry (DSC) was used to determine the T_m for DPPC membranes in the presence of the dye and the borates. Addition of Cy^{3+}, Ph_3BnB^-, and Ph_4B^- in the relative amounts used in the bleaching and quenching experiments described above lowered the phase transition temperature from 41.4 to 39.6 °C (Figure 8).

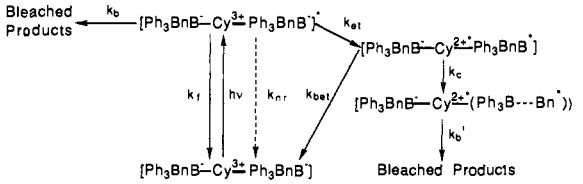
Electron transfer from Ph_3BnB^- to excited Cy^{3+} effectively quenches the fluorescence of the dye. This process was also studied

Scheme I[a]



[a] Charges have been omitted within the complexes for simplicity.

Scheme II



in DPPC liposomes as a function of temperature. From Figure 9 the quenching is clearly more efficient at temperatures below the T_m than above. On the basis of the slopes of the lines for the $T = 25$ °C and the $T = 55$ °C data, the process is more favorable when the membrane is in the gel state by a factor of 6.5.

Discussion

Schuster and co-workers have conducted extensive photochemical and photophysical studies involving organic solutions of cyanine dyes and borates such as **II**.[39,40] One interesting result was that even in benzene, where cyanine and borate were associated as contact ion pairs, no perturbation of the dye absorption spectrum was observed. Very different behavior is observed in water, however, for Cy^{3+} and Ph_3BnB^- (Figure 1). The spectral effects observed upon addition of Ph_3BnB^- to Cy^{3+} in water, i.e., broad, red-shifted absorbance and a diminished extinction coefficient, are typically ascribed to charge-transfer (CT) interaction.[41,42] However, other observations argue against CT stabilization of the complex. Generally, weaker reductants form weaker CT complexes exhibiting shorter λ_{max}. However, nearly identical spectral perturbations are observed when Cy^{3+} is titrated with either Ph_4B^- or Ph_3BnB^-, although Ph_4B^- is a weaker reductant than Ph_3BnB^- by 360 mV.[39] Also, CT complexes are usually only weakly associated. The binding phenomenon illustrated in Figure 1 is quite strong, occurring with very little added borate. The complexation observed in aqueous media appears to be primarily driven by the hydrophobicity of the borate. In acetonitrile, where Ph_3BnB^- is soluble, no complexation is observed with Cy^{3+} even when a 500-fold excess of borate has been added.

It is interesting that in water all three dyes investigated form complexes with Ph_3BnB^- having a net charge of +1. These complexes are formed readily but appear to strongly resist the addition of one more borate. For example, Cy^{3+} adds up to 2.0 equiv of Ph_3BnB^- with clean isosbestic behavior, which indicates formation of a single complex with a 1:2 stoichiometry (Figures 1 and 2). Upon further addition of borate, isosbestic behavior is lost, yet the complex remains solubilized. It seems unlikely that a 1:3 complex, which would be formally uncharged, could remain dissolved in water. Rather, it is more plausible that oligomerization of existing 1:2 complexes is mediated by excess borate (Scheme I). The formation of larger structures is also suggested by the increased light scattering observed at shorter wavelengths as excess (>2 equiv) borate is added.

The cyanine–borate complexes exhibit some unusual photophysical and photochemical properties. The pathways open to the excited complexes are illustrated in Scheme II. Four processes are considered: (1) direct reaction of the complex to yield bleached dye (k_b); (2) fluorescence (k_f); (3) nonradiative decay, which may consist of several pathways (k_{nr}); and (4) intracomplex electron transfer from borate to dye (k_{et}). Full charges are assigned to the components of the complex because there is no evidence for charge-transfer interaction (vide supra). Since the fluorescence of the dye within the complexes is strongly quenched but relatively slow bleaching is observed, the first two processes must represent minor modes of deactivation for the excited complex. As mentioned above, excited cyanines typically decay nonradiatively by twisting about the methine bridge linking the two heterocyclic ends of the dye.[30-32] However, this motion is likely to be severely restricted by the strongly bound borate molecules, reducing the contribution of this process to deactivation. Finally, the fluorescence quenching results (Table II) imply that electron transfer within the complex is a significant decay path. Stronger quenching is observed for the Ph_3BnB^- complex than for the analogous Ph_4B^- complex, an effect attributable to the lower oxidation potential of Ph_3BnB^-.

Although bleaching for either borate complex is fairly slow and represents a minor pathway, the drastically different behavior exhibited by the two complexes in this regard warrants further comment. It seems logical that bleaching would occur from a point in the mechanism where the complexes are significantly different. Within Scheme II, such a situation arises only after electron transfer from borate to dye within the excited complex (k_{et}). Schuster and co-workers have established that oxidation of this class of borates by PET produces a boranyl radical which is capable of decomposing to form triphenylboron and a radical, R•, by cleavage of a boron–carbon bond.[43,44] When the borate is Ph_4B^-, an unstable phenyl radical would be produced. In this case, back electron transfer is a much more favorable process than bond cleavage ($k_{bet} \gg k_c$). However, when the borate is Ph_3BnB^-, the resonance-stabilized benzyl radical produced would allow decomposition to compete effectively with back electron transfer. Clearly, the path followed after electron transfer depends very strongly on which borate is present in the complex. Thus, bleaching is believed to occur after electron transfer within the excited complex as this leads to a point in the mechanism at which the complexes are distinctly different.

The photobleaching and quenching results obtained for Cy^{3+} and borate in the presence of liposomes are more straightforward. Cy^{3+} clearly binds to the surface of anionic DOPC/DOPA liposomes as evidenced by the large bathochromic shift in the absorption spectrum (Figure 4) and enhanced fluorescence yield from the dye in the presence of the liposomes. Kunitake has reported similar spectral evidence for binding of anionic cyanines to cationic membranes.[37,45] Since the absorption profile is unchanged upon binding (aside from the red shift), it is concluded that the dye is monomeric when bound to the liposome surface. Addition of borate has very little effect on the spectrum, so that complexation does not occur within the membrane. Irradiation leads to extensive bleaching of the dye only when Ph_3BnB^- is present. If no borate or only Ph_4B^- is present, the dye is stable to irradiation (Table II). Moreover, the fluorescence of the dye is quenched by Ph_3BnB^- but not Ph_4B^- when bound to liposomes. These results indicate that excitation of the dye leads to electron-transfer quenching by Ph_3BnB^-, and subsequent decomposition of the boranyl radical competes favorably with back electron transfer, leading to irreversible bleaching of the dye.

As mentioned in the introduction, the phospholipid membrane represents a medium whose properties can be varied with relative ease. Experiments involving membranes having an accessible gel–liquid crystalline phase transition temperature are particularly attractive since this permits study of membrane-bound processes in either a solidlike or liquidlike environment. Substantial differences were expected for the PET reaction between Ph_3BnB^- and Cy^{3+} in the two phases.

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DPPC was chosen for these experiments because its T_m (41.4 °C) provides a convenient temperature range in which to utilize both the gel and liquid crystalline phases. The dye, when bound to DPPC liposomes by borate, bleached rapidly, analogous to the results for DOPC liposomes. In order to prevent release of the dye as the reaction proceeded, a large excess of Ph_4B^- was used as an inert binding agent. This minimizes the ambiguity in the average donor–acceptor separation distance by keeping the dye bound to the liposome at all times. Efficiency of the PET reaction between Cy^{3+} and Ph_3BnB^- in DPPC liposomes was studied as a function of temperature for both bleaching and fluorescence quenching experiments. Both processes proved to be more efficient when the membrane was below its phase transition temperature (39.6 °C in the presence of the dye and the borates). Bleaching (Figure 7) and quenching (Figure 9) were 3.5 and 6.5 times more efficient, respectively, below the phase transition. (Initially this difference was ascribed to back electron transfer. However, as described below, back electron transfer appears to be a very minor pathway in this system.) The discontinuity observed in Figure 7 establishes that the effect observed is not merely attributable to changing temperature, but rather is due to the abrupt change in the physical properties of the membrane at its phase transition. In the gel state, the membrane presents a viscous medium which retards the diffusion of molecules solubilized within the bilayer. Fayer and co-workers have recently investigated the effect of diffusion on electron-transfer reactions.[46] Their results clearly demonstrate that electron transfer is more efficient in fluid media than in solid media. However, the efficiency of electron transfer from the membrane-bound borate to Cy^{3+} is enhanced in the less fluid gel state. Therefore we conclude that this interesting effect is not due to restricted diffusion. Another possible explanation for the observed results is that the excited-state lifetime of Cy^{3+} is longer in the gel state than in the liquid crystalline state. This would permit more efficient PET, reflected in a greater extent of fluorescence quenching as illustrated in Figure 9. Assuming the radiative rate constant k_f has the same value above and below T_m, an estimate for the ratio of the lifetimes in the two states can be obtained from the ratio of the fluorescence yields as follows:

$$\frac{\Phi_f^{gel}}{\Phi_f^{lc}} = \frac{I_f^{gel}}{I_f^{lc}} = \frac{k_f^{gel} * \tau^{gel}}{k_f^{lc} * \tau^{lc}} \approx \frac{\tau^{gel}}{\tau^{lc}}$$

(I_f is the experimentally determined integrated emission spectrum of Cy^{3+}.) This ratio is 1.6, which is substantially less than the factor of 6.5 observed for the overall efficiency of the quenching processes. As shown below, the difference in the quenching efficiencies in the two states cannot be attributed solely to increased lifetime in the gel state. The data indicate that the rate constant for quenching is also greater below T_m than above:

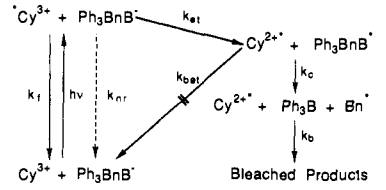
$$\frac{\Phi_0}{\Phi} = 1 + K[Q] = 1 + k_q\tau[Q]$$

$$\frac{K^{gel}}{K^{lc}} = 6.5 = \frac{k_q^{gel} * \tau^{gel}}{k_q^{lc} * \tau^{lc}} = \left(\frac{k_q^{gel}}{k_q^{lc}}\right)(1.6)$$

$$\frac{k_q^{gel}}{k_q^{lc}} = 4.1$$

A plausible explanation for the enhanced electron-transfer quenching below T_m is a smaller donor–acceptor distance of separation in the gel phase than in the liquid crystalline phase. This could occur from a change of position of the borate alone, of the dye alone, or of both the dye and the borate. The narrow absorption profile of Cy^{3+} when bound to DPPC liposomes suggests the dye is not experiencing a variety of environments but rather occupies a well-defined binding site within the membrane. Moreover, the absorption spectrum is independent of the physical state of the membrane. As shown in Figure 5, the absorption λ_{max} of Cy^{3+} is very sensitive to the dielectric of the medium. Since there is a fairly steep dielectric gradient across the membrane interface,[21] from bulk water (ϵ = 78) to bulk hydrocarbon (ϵ = 2–3), any change in the binding site of the dye within the membrane would be accompanied by a shift in the absorption spectrum. As no shift is observed upon a change of temperature, we conclude that the dye is bound at the same membrane site both above and below T_m. Therefore, the position of the borate within the membrane must be changing as the sample temperature changes. According to this model, the borate would be closer to the surface of the membrane in the gel state than in the liquid crystalline state. Recent studies place Ph_4B^- 3–5 Å below the ester carbonyl of the more deeply penetrating chain in DPPC membranes, locating the borate in the hydrocarbon tail region of the bilayer.[18] (The possibility of different binding sites above and below the phase transition was not considered in that study.) Ph_3BnB^-, because of its close structural similarity to Ph_4B^-, is expected to occupy a similar binding site. In the gel state, the lipids are packed closely together in an essentially crystalline lattice, whereas the medium is considerably more fluid above T_m. Therefore a bulky molecule such as Ph_3BnB^- would certainly have a more difficult time penetrating into the hydrocarbon tail region when the membrane is below the phase transition, leading to a binding site closer to the surface of the liposome. This would result in a more efficient PET from Ph_3BnB^- to Cy^{3+} below the phase transition.

Scheme III



The similarity between the relative efficiencies of photobleaching and fluorescence quenching in the two states of the bilayer (3.5 and 4.1, respectively) indicates that back electron transfer is not an important pathway in the system (Scheme III). This is in accord with the results obtained by Schuster in which even in highly viscous solvents back electron transfer could not compete with carbon–boron bond cleavage in the oxidized borate when a stabilized radical could be produced.[39] This has significance with respect to the question of energy storage. In the absence of back electron transfer, the energy which remains in the system after excitation and electron transfer should be harvestable with reasonable efficiency. Moreover, redox potentials obtained in homogeneous solution suggest that the forward electron transfer is only slightly exergonic.[49] Thus, very little of the initial excitation energy is lost in the transfer of an electron from Ph_3BnB^- to Cy^{3+}. These two features indicate that the system is capable of storing a significant amount of the excitation energy for a useful amount of time. Future experiments will be directed toward actually harvesting this energy, as well as extending the sensitivity of the system to other regions of the visible spectrum.

One final point of discussion involves comparison of the results obtained in DPPC liposomes in this study with those mentioned in the introduction. The work by Ottolenghi and associates[12,47] as well as by Kano et al.[13] demonstrated enhanced electron transfer above the phase transition for donor and acceptor solubilized within the bilayer, whereas we have found the opposite effect. The previous results were interpreted to be due to diffusion-controlled electron-transfer reactions. In those studies, it is likely that the donor and acceptor binding depths within the membrane overlapped at all temperatures. Under such circumstances both

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(48) *Lange's Handbook of Chemistry*, 13th ed.; Dean, J. A., Ed.; McGraw-Hill: New York, 1985.
(49) $E_{ox}(Ph_3BnB^-)$ = 1.09 V from ref 39. $E_{red}(Cy^{3+})$ has not been determined. However, the reduction potential for the *N,N'*-diethyl analogue is −0.885 V.[50]
(50) Parton, R. L.; Lenhard, J. R. *J. Org. Chem.* **1990**, *55*, 49–57.

diffusion and electron transfer occur *laterally* within the membrane. The results reported here describe a system where the donor and acceptor are bound at different depths within the membrane. Thus, while diffusion is still primarily lateral in nature, electron transfer occurs in the *transverse* direction. It is the vectorial arrangement of the system which permits the distance change in passing through the phase transition to overcome the effects of diffusion, allowing the exponential dependence of the reaction on distance to become the dominant factor. This results in the opposite dependence of the PET reaction on the membrane physical state from that reported in the earlier work.

In conclusion, liposomal membranes have been used as media for photoinduced electron transfer reactions. The donor and acceptor were conveniently separated by binding at different depths within the bilayer membrane. This is particularly important for hydrophobic molecules such as the borates employed in this study. The aqueous complexes formed by these anions with water-soluble cationic dyes have intricate photophysical and photochemical pathways open to them, resulting in relatively inefficient PET. Solubilization within phospholipid membranes prevents this association, thereby reducing the number of pathways open to the excited cyanine and enhancing PET from borate to dye. Moreover, the donor-acceptor separation and, therefore, electron-transfer efficiency are dependent on the physical state of the membrane. The strong distance dependence of PET reactions leads to dramatic effects on the photobleaching and fluorescence quenching efficiencies. In fact, these types of experiments may provide a means of accurately determining the locations of membrane-bound donors and acceptors, provided the binding site of one of the redox partners is known and one can quantitatively account for diffusion and the variable dielectric. An additional appealing feature of the system is that back electron transfer appears to be a very minor process within the membrane. This permits the liposomal system to store the excitation energy as redox products for potentially useful amounts of time. This general strategy could in principle be used to generate a large number of reduced acceptors at or near the surface of a liposome. The stored energy could then be used to drive subsequent chemical transformations, a system which is made even more attractive by the high surface area afforded by liposomes. In fact, Willner and associates have incorporated many of these features into a colloidal-based system which effectively hydrogenates ethylene.[16] However, in that system the chemical processes were confined to the colloid surface or bulk aqueous medium, whereas the system described here is based on redox processes occurring within the membrane itself. Finally, the results obtained in this study suggest that the design of membrane-based systems for the harvest and conversion of light energy should include an investigation of the role of the lipid bilayer in promoting or inhibiting the desired photochemistry.

Acknowledgment. The authors thank the National Science Foundation for partial support of this research.

Kinetic Deuterium Isotope Effects as Evidence for a Common Ion-Pair Intermediate in Solvolytic Elimination and Substitution Reactions of 1,1-Diphenylethyl Chloride

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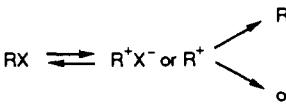
Abstract: Solvolysis of 1,1-diphenyl-1-chloroethane (**1**-Cl) in acetonitrile at 25 °C provides the elimination product 1,1-diphenylethene (**3**). Addition of water or methanol to the acetonitrile increases the rate of elimination and gives rise to a second product, 1,1-diphenyl-1-hydroxyethane (**1**-OH) or 1,1-diphenyl-1-methoxyethane (**1**-OMe). The deuteriated analogue, 1,1-diphenyl-1-chloro[2,2,2-$^2H_3$]ethane (d-**1**-Cl), reacts slower. The overall kinetic deuterium isotope effect was found to decrease from $(k_{12}^H + k_{13}^H)/(k_{12}^D + k_{13}^D) = 1.73$ in pure acetonitrile to 1.34 in 20 vol % methanol in acetonitrile. This isotope effect is composed of a substitution isotope effect k_{12}^H/k_{12}^D and an elimination isotope effect k_{13}^H/k_{13}^D, which simultaneously change from 0.84 to 0.96 and from 2.2 to 3.2, respectively, when the methanol concentration is increased from 1.96 to 9.09 vol %. These results indicate a branched mechanism involving an almost rate-limiting formation of a common carbocationic intermediate. The acid-catalyzed solvolysis of 1,1-diphenyl-1-phenoxyethane (**1**-OPh) in acetonitrile containing 0.4 vol % aqueous 2 M sulfuric acid quickly provides the alcohol **1**-OH, $k_{12}/k_{13} \approx 11$, which in a subsequent fast reaction yields the olefin **3** as the final product. In contrast, the solvolysis of the chloride **1**-Cl in 0.4 vol % water in acetonitrile yields almost exclusively olefin **3**, $k_{12}/k_{13} = 0.003$. The results strongly indicate that the leaving chloride anion is involved in the elimination process and that the common intermediate is the contact ion pair. The isotope effect of the acid-catalyzed elimination of the alcohol **1**-OH in 0.4 vol % water in acetonitrile is $k_{13}^H/k_{13}^D = 5.3$, which increases to $k_{13}^H/k_{13}^D = 6.5$ in 25 vol % acetonitrile in water. The possibility of an ion-molecule pair intermediate in the acid-catalyzed solvolysis is discussed.

Introduction

Solvolytic reactions of substrates having at least one hydron in the β position generally give both a substitution product, by reaction with the solvent or added nucleophile, and an elimination product. The substitution product is usually the dominant product in nucleophilic solvents. When the experimental results indicate stepwise solvolysis, the substitution and the elimination have frequently been postulated for the sake of mechanistic simplicity to occur via a common carbocationic intermediate (eq 1). Kinetic deuterium isotope effects for the separate reactions have provided experimental evidence for branching via a common ion pair in the solvolysis of the relatively unreactive 9-(2-chloro-2-propyl)-fluorene[1] in a highly aqueous medium and strong support for branching in some other systems.[2-5]



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