Lipid Bilayer Enhanced Photoinduced Electron Transfer

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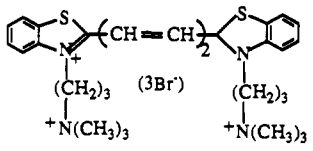
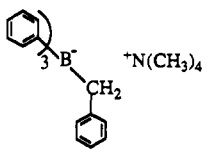
Microheterogeneous media, such as colloids, zeolites, micelles, and liposomes, are frequently effective in enhancing photochemical reactivity over that observed in homogeneous solution.[1] The distinct hydrophobic and hydrophilic regions inherent in liposomes make them particularly attractive systems for controlling the reactivity in processes such as photoinduced electron transfer (PET).[2,3] Generally the liposome studies have focused on electron transfer across bilayer membranes.[4] Some important examples of efficient electron transfer from neutral photoexcited donors within the bilayer to charged acceptors at the surface have appeared.[5,6] Recently Willner and associates introduced the use of charged interfaces to effectively prevent the complex formation of oppositely charged donors and acceptors.[7] In the present study we extend that concept by the use of a lipid-bilayer interface to enhance PET by preventing complex formation of a tricationic cyanine (I) and the hydrophobic triphenylbenzylborate anion (II). Schuster and co-workers recently demonstrated efficient PET from similar anionic triphenylalkylborates to cationic cyanine dyes in low-polarity organic solvents.[8]

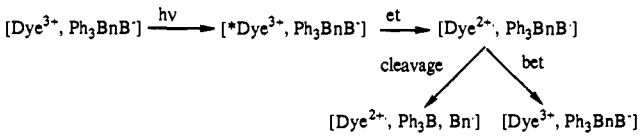


I



II

In water, the absorption spectrum of I (λ_{max} = 649 nm) shifts to the red (678 nm) upon titration with **II** (Figure 1A, curves a and b) due to formation of a strong complex, driven by the hydrophobicity of the borate.[9] Irradiation of the complex with red light slowly bleaches the dye (Figure 1A, curve c).[10] Complex formation between donor and acceptor diminishes the overall efficiency of PET because of the rapid, highly exergonic back reaction. The observed net photochemistry in this case is attributable to decomposition of the incipient boranyl radical, which competes with back electron transfer (BET) as shown below.[8]



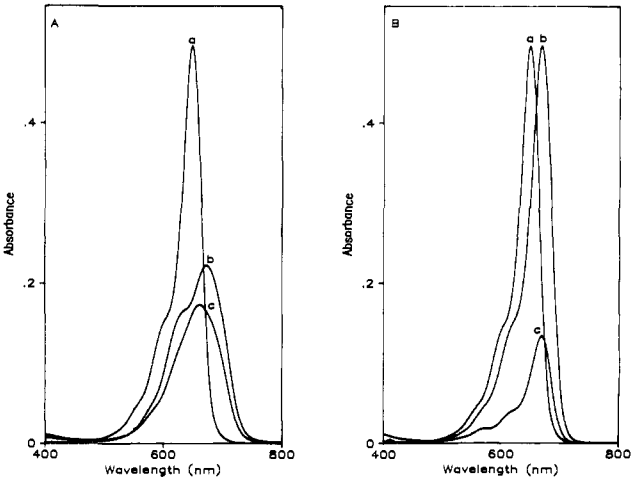


Figure 1. (**A**) Absorption spectra of 3.0 μM dye **I**: (a) in water, λ_{max} = 649 nm; (b) after addition of 7.2 μM **II**; and (c) after red light irradiation of sample b for 10 min. (**B**) Absorption spectra of 3.0 μM dye **I**: (a) in water, λ_{max} = 649 nm; (b) in an aqueous suspension of DOPC/DOPA (9:1) liposomes (0.5 mg/mL) with 7.2 μM **II**, λ_{max} = 668 nm; and (c) after red light irradiation of sample b for 10 min.

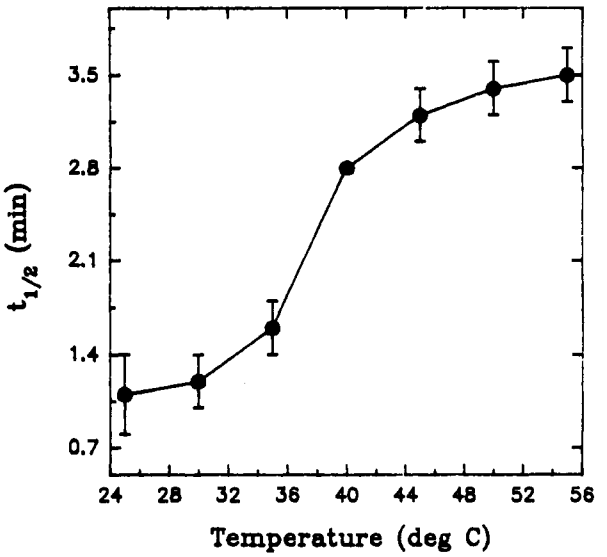


Figure 2. Effect of temperature on the half-life rate of 1.38 μM **I** during red light photobleaching in the presence of 4.15 μM **II** in DPPC liposomes (0.5 mg/mL).

Quite different results are obtained in liposomes. Addition of **I** and **II** to anionic liposomes composed of DOPC/DOPA (9:1)[11] causes a significant red shift of the entire absorption profile due to the binding of **I** to the bilayer surface (Figure 1B, curves a and b). Irradiation of the liposomal system rapidly bleaches the dye (Figure 1B, curve c). The dye and borate are apparently close enough to one another, on average, to permit facile forward electron transfer yet far enough apart to allow decomposition of the boranyl radical to compete effectively with BET.

Tetraphenylborate, a close structural analogue of **II**, is known to partition overwhelmingly[12] from aqueous media into the hydrophobic interior[13] of phospholipid membranes. Therefore, borate

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(2) For a review of the work with micelles, see ref 1, pp 104-115.
(3) For a review of the studies with liposomes, see: Baral, S.; Fendler, J. In *Photoinduced Electron Transfer*: Park B; Fox, M. A., Chanon, M., Eds.; Elsevier; Amsterdam, 1988.
(4) For a review, see: Robinson, J. N.; Cole-Hamilton, D. J. *Chem. Soc. Rev.* **1991**, *20*, 49-94.
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(8) (a) Chatterjee, S.; Gottschalk, P.; Davis, P. D.; Schuster, G. B. *J. Am. Chem. Soc.* **1988**, *110*, 2326-2327. (b) Chatterjee, S.; Davis, P. D.; Gottschalk, P.; Kurz, M. E.; Sauerwein, B.; Yang, X.; Schuster, G. B. *J. Am. Chem. Soc.* **1990**, *112*, 6329-6338.
(9) The complex appears to have very little charge-transfer character. Titration of I with Ph_4B^- produces an identical spectral change despite the fact that the oxidation potential of Ph_4B^- is 360 mV higher than that of **II**.[8b]
(10) All irradiations were performed in a quartz cuvette (22.4 cm from the lamp) with light from a 200-W Hg(Xe) arc lamp, which was filtered through CS2-62 (Corning Glass Works, λ > 580 nm) and neutral density (6% transmitting) filters.
(11) Liposomes of dioleoylphosphatidylcholine (DOPC) and dioleolylphosphatidic acid (DOPA) were prepared in 10 mM imidazole buffer (pH = 7.0), by freeze-thawing followed by extrusion through 0.1-μm Nuclepore filters.
(12) The equilibrium constant favors the bilayer vs water by a factor of 10^5: (a) Brock, W.; Stark, G.; Jordan, P. C. *Biophys. Chem.* **1981**, *13*, 329-348. (b) Bruner, L. J. *J. Membr. Biol.* **1975**, *22*, 125-141. (c) Smejtek, P.; Wang, S. *Biophys. J.* **1991**, *59*, 1064-1073.

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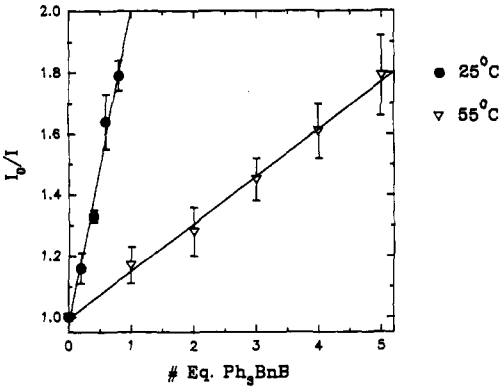

Figure 3. Effect of temperature on the fluorescence quenching of 0.27 μM I by II in DPPC liposomes (0.1 mg/mL).

II alone should render the liposome anionic, removing the need for an anionic phospholipid. In fact, addition of I and II to pure DOPC liposomes followed by irradiation yields results very similar to those obtained with anionic liposomes.

The effect of the physical state of the lipid bilayer on PET between I and II was studied using dipalmitoylphosphatidylcholine (DPPC) liposomes. The time required to bleach 50% of the dye was measured over a 30 °C temperature range (Figure 2). The reaction is 3.5 times faster at 25 °C than at 55 °C, with the rate changing significantly at the phase-transition temperature (T_m) of the membrane.[14] Figure 3 shows that the fluorescence quenching of the dye by the borate in DPPC liposomes is also more efficient (by a factor of 6.5) below T_m. Whereas the photobleaching experiment probes the net PET reaction, fluorescence quenching allows study of the forward reaction separate from the back transfer. The greater sensitivity to temperature observed in the emission data compared to the photobleaching reaction suggests that BET is also favored below the T_m.

A reasonable explanation for these results is suggested by the greater disorder of the acyl chains in the L_α phase above the T_m, which could allow a deeper penetration of II into the bilayer, with two important ramifications. First, the donor–acceptor distance of separation increases, and second, the borate moves deeper into a low dielectric constant region. Both factors would retard the rate of electron transfer,[15] consistent with the data shown in Figures 2 and 3.[16]

In conclusion, the net photoinduced electron transfer from triphenylbenzylborate (II) to water-soluble cyanine I is greatly enhanced by binding of both reactants to PC bilayers. The photoredox reaction involves transfer of an electron from the hydrophobic interior of the bilayer to its surface. The efficiency of the process is dependent on the physical state of the bilayer. A significant amount of the excitation energy is stored in the form of the reduced dye and benzyl radical liberated by decomposition of the oxidized borate. Current efforts are directed toward utilizing this energy to drive subsequent chemical reactions.

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

(13) Estimates for the location of tetraphenylborate in phospholipid bilayers place it slightly (<5 Å) below the ester carbonyls in the lipid tails. See ref 12c and the following: Flewelling, R. F.; Hubbell, W. L. *Biophys. J.* **1986**, *49*, 541–552.

(14) The T_m for DPPC is shifted from 41.4 °C to 39.7 °C upon addition of I and II in the relative amounts used in the photobleaching and quenching experiments. Differential scanning calorimetry was performed with a Microcal MC-2 calorimeter at a scan rate between 10 and 15 deg/h.

(15) Marcus, R. A. *J. Chem. Phys.* **1956**, *24*, 966–978. Marcus, R. A. *J. Chem. Phys.* **1957**, *26*, 867–871. Marcus, R. A.; Sutin, N. *Biochim. Biophys. Acta* **1985**, *811*, 265–322.

(16) Calculations based on the fluorescence quenching data and considering only the effect of distance on electron-transfer rates indicate that the borate moves no further than 2 Å deeper into the bilayer above T_m. The actual distance is likely to be smaller when the dielectric constant of the medium is taken into account.

A Chemical Model for Phosphomutases: A Dissociative Thiophosphoryl Transfer Reaction Proceeding with Retention of Configuration

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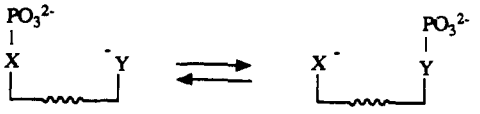
Phosphomutases catalyze an apparent intramolecular phosphoryl transfer reaction, eq 1. We report here a simple chemical model for a dissociative mutase-like reaction and demonstrate that this proceeds with overall excess retention of configuration despite the fact that only a single thiophosphoryl transfer is involved.



(1)

Phosphomutases have been shown to catalyze reactions with overall retention of configuration.[1] This observation is explicable in terms of a double displacement involving a bis-phosphorylated cofactor for one class of such enzymes and for the other class a phosphoenzyme intermediate. The question of whether retention of configuration is a *necessary and sufficient* observation from which to conclude a double displacement (two phosphoryl transfers) is an intriguing one.

The majority of enzyme-catalyzed phosphoryl transfer reactions, in particular kinases, have been shown to proceed with inversion of configuration.[2] In contrast, mutases and some phosphatases have been shown to catalyze phosphoryl transfer with overall retention of configuration. In many of these cases there is good independent evidence for a double-displacement mechanism. The conclusion therefore must be that single phosphoryl transfer steps occur with in-line geometry. The debate over whether individual phosphoryl transfer steps are formally associative or dissociative has rumbled on, in part because model studies demonstrate that although monomeric metaphosphate is not an intermediate in such reactions in dilute aqueous solutions,[3] it does participate in reactions in a variety of organic media including *tert*-butyl alcohol,[4] and it is not clear how the active sites of proteins should be viewed in terms of the environment that they provide for the reaction. Indeed it is tantalizing to imagine the functional significance of the movement of "hinged" regions of proteins (common in phosphotransferases) that effectively sequesters the substrate (and intermediates) away from water. In this present study we have probed the stereochemical constraints on the dissociative pathway and have developed a simple chemical model for a phosphomutase-type reaction.

(R_p)-2-(Hydroxymethyl)-4-nitrophenyl [^{16}O, ^{18}O]thiophosphate (1) can be readily synthesized by our published route.[5] We have deliberately chosen to study a thiophosphoryl transfer reaction since we have confidence that in alcohols this type of reaction is fully dissociative.[6] Furthermore, our stereochemical analysis is

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