Enhancement of Energy Transfer on Bilayer Surfaces via Polymerization-Induced Domain Formation

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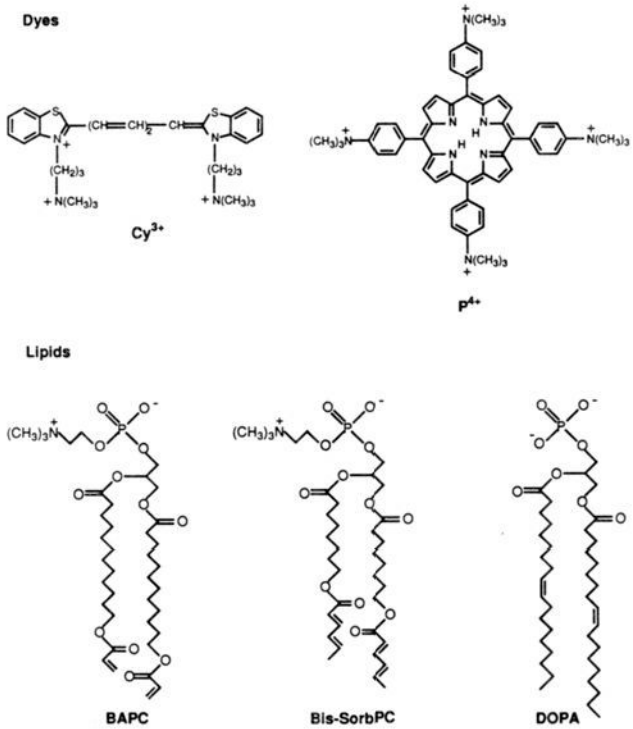
Received May 10, 1993

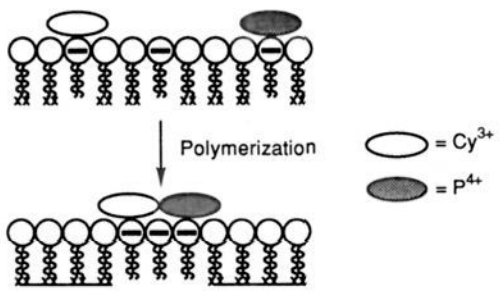
Photosynthetic plants and bacteria utilize electronic energy-transfer and photoinduced electron-transfer reactions for the collection, conversion, and storage of light energy.[1,2] Elaborate membrane-bound systems consisting of a variety of cofactors incorporated into a transmembrane protein matrix are the reaction centers for this chemistry. The proteins provide vital structural support to the system by binding the cofactors at well-defined distances and orientations with respect to one another, creating high effective concentrations.[3-5] In the absence of the protein, the cofactors would be free to diffuse throughout the lipid bilayer membrane, seriously diminishing the conversion efficiency.

The use of liposomes as model systems as well as for the development of synthetic photochemical molecular devices[6] requires a strategy for achieving high effective concentrations of the redox and energy-transfer "cofactors".[7-9] One approach involves covalent linkage of the various components by chemical synthesis, thereby forming a molecular wire for insertion into a lipid bilayer.[10-13] An alternative approach described here is to render a significant percentage of the membrane inaccessible to the bound cofactors. This was accomplished by the combination of (a) polymerization-induced domain formation within a two-component lipid bilayer and (b) targeting of the cofactors to the resulting unpolymerized domains. Polymerization-induced domain formation has previously been usefully employed to form lipid domains for the insertion of proteins into polymerized bilayers[14] and to destabilize bilayers.[15] The model system studied here involves energy transfer (ET) from a porphyrin (P^{4+}) to a cyanine dye (Cy^{3+}) (see Chart I for structures), both electrostatically associated with the liposome surface. This reaction has also been used to sensitize[16] a vectorial photoinduced electron transfer in bilayers.[17,18] As shown in Scheme I (only the bilayer outer leaflet is shown for simplicity), polymerization triggers domain formation in the bilayer. By using an anionic lipid (dioleoylphosphatidic acid, DOPA) as the nonpolymerizable component, the two pigments selectively bind to the unpolymerized domains. The shorter average distance of separation after polymerization results in enhanced ET.

Chart I



Scheme I



The liposomes were composed of a 9:1 ratio of polymerizable lipid to DOPA. Complete polymerization renders as much as 90% of the liposome surface inaccessible to P^{4+} and Cy^{3+}, since DOPA should not diffuse into the cross-linked polymeric domains. Approximately 800 DOPA molecules are in each liposome outer leaflet; therefore, dye saturation of the surface sites was not encountered.

Excitation of BAPC/DOPA-bound P^{4+} at 417 nm results in the characteristic double-peaked fluorescence.[19] Successive addition of Cy^{3+} in increments of 20 per liposome quenches the P^{4+} emission at 650 nm and sensitizes the Cy^{3+} fluorescence at 696 nm (Figure 1). The clean isoemissive point at 661 nm indicates that quenching occurs solely by energy transfer.

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(19) BAPC/DOPA (9:1) and bis-SorbPC/DOPA (9:1) liposomes were prepared in Milli-Q water by freeze–thawing followed by extrusion through 0.1-μm Nuclepore filters. Polymerizable lipid concentration was determined from the UV absorption.

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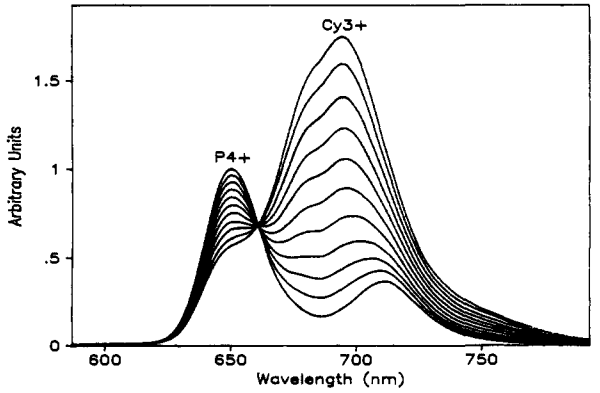

Figure 1. Fluorescence spectra (excitation at 417 nm) for P^{4+} (25.0 nM, 10 per liposome) in the presence of unpolymerized BAPC/DOPA (9:1) liposomes (in water), with addition of Cy^{3+} in increments of 20 cyanines per liposome.

A dimensionless quantity R, defined as the ratio of the emission intensities at 696 and 650 nm, increases as ET becomes more efficient. Figure 2 illustrates the change in R with added Cy^{3+} for BAPC/DOPA (9:1) liposomes polymerized to different extents.[20] The more rapid increases in R in the polymerized relative to the unpolymerized liposomes indicates that energy transfer is clearly enhanced in the polymerized liposomes. The initial slopes (from 0 to 60 Cy^{3+} per liposome) were used to estimate the ET quantum efficiencies: from 0.09 before polymerization to 0.27 and 0.71 after 50% and 100% polymerization of the BAPC, respectively.[21] Similar results were found for bis-SorbPC/DOPA (9:1) liposomes, which were 90% polymerized under conditions identical to those used for BAPC/DOPA.[20] Energy transfer from P^{4+} to Cy^{3+} was enhanced 8-fold as a consequence of the polymerization.

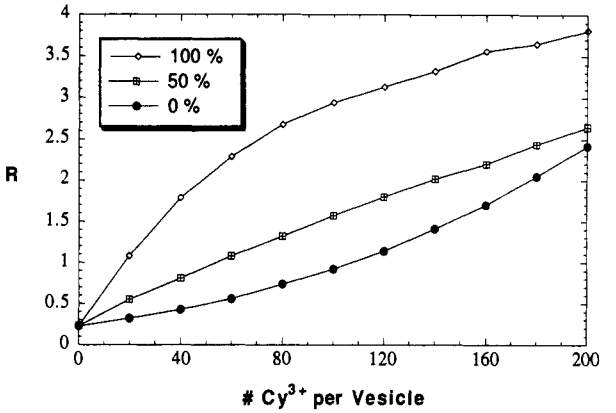

Figure 2. Variation of R (defined in the text) with the number of Cy^{3+} bound per BAPC/DOPA (9:1) liposome polymerized to different extents (2.5 nM liposomes and 25.0 nM P^{4+}). The data for 0% polymerization are the average of two separate trials; the other data are for single experiments.

In conclusion, polymerization-induced domain formation in lipid bilayers is a useful strategy for increasing the effective concentrations of membrane-bound donor and acceptor molecules, as shown by the enhanced energy transfer reported here. Future work will focus on application of this approach to improving the efficiency of membrane-based vectorial photoinduced electron-transfer systems described previously.[17,18]

Acknowledgment. We warmly thank Henry Lamparski for a generous gift of bis-SorbPC and for advice regarding the preparation of BAPC. Partial support of this research by the National Science Foundation is greatly appreciated.

(20) Liposome polymerization was initiated with AIBN: [lipid]/[AIBN] was 10. The AIBN was decomposed (under argon) using the filtered output ($\lambda > 345$ nm) of a 200-W Hg(Xe) arc lamp. For BAPC/DOPA, approximately 50% polymerization was achieved by 8-h irradiation, while complete conversion occurred in 14 h. Bis-SorbPC/DOPA was 90% polymerized after 6 h of irradiation.

(21) The fluorescence lifetime, τ_F, of P^{4+} in H_2O is 9.3 ns.[22] The Φ_F of P^{4+} was enhanced 10–15% on liposome binding; therefore, if k_F is the same in H_2O and liposomes then the τ_F for bound P^{4+} is ca. 10.5 ns. The slope of a Stern–Volmer fit of data is 15.6 M^{-1}, which when combined with τ_F yields a k_{ET} of 1.5×10^9 M^{-1} s^{-1}. This value permits the calculation of the quantum efficiency for energy transfer, 0.09, in the unpolymerized liposomes.

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