# Excitation energy transfer in *Rhodopseudomonas sphaeroides* chromatophore membranes fused with liposomes

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The role of phospholipid in the structural organization of the light-harvesting complexes of *Rhodopseudomonas sphaeroides* was examined in photosynthetic (chromatophore) membrane vesicles fused with liposomes. Photochemically active preparations with progressive phospholipid enrichment up to >15-fold were obtained by both polyethylene glycol- and acidic-pH-induced fusion. Their fluorescence emission at ~300 and 77 K was increased by 2–3.5-fold from the peripheral B800–850 antenna relative to that from the core B875 antenna. Up to 30–40% reduction in the efficiency of excitation energy transfer between B850 and B875 was also observed at 77 K suggesting a selective, phospholipid-induced dissociation of a portion of the B800-850 from the rest of the light-harvesting system.

*Bacterial photosynthesis* *Bacteriochlorophyll-protein complex* *Fluorescence spectroscopy* *Light-harvesting complex* *Liposome fusion* *Photosynthetic membrane* (Rhodopseudomonas sphaeroides)

## 1. INTRODUCTION

The intracytoplasmic (chromatophore) membrane of the photosynthetic bacterium *Rhodopseudomonas sphaeroides* contains peripheral and core light-harvesting pigment-protein complexes designated B800-850 and B875, respectively, from their near-infrared absorption maxima [1,2]. Fluorescence yield [3,4] and singlet-triplet annihilation [5] studies have suggested that radiant energy harvested in 'lakes' of the B800-850 antenna is transferred to B875 which surrounds, interconnects and serves as the immediate energy donor to the reaction center phototraps. This is supported by the order in which the pigment-protein complexes are assembled [6] as well as from their behavior in LDS-polyacrylamide gel electrophoresis at 4°C [2].

Despite these proposed associations, the precise role of protein-protein and phospholipid-protein interactions in excitation energy migration within the chromatophore membrane remains unknown. Such phospholipid-protein associations have been suggested from studies with fluorescent [7] and spin-labeled [8] probes. It was proposed that protein within the bilayer significantly restricts the motion of fatty acyl chains [7] and that negatively charged phospholipids associate with B800-850 [8]. Apparent phospholipid-B800-850 associations have also been demonstrated in LDS-polyacrylamide gel electrophoresis [9].

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*Abbreviations:* BChl, bacteriochlorophyll *a*; B800-850, B875, peripheral and core light-harvesting BChl-protein complexes, respectively, identified by near-infrared absorption maxima; LDS, lithium dodecyl sulfate; PEG, polyethylene glycol 6000; P870, reaction center BChl absorption band which functions as the primary electron donor species and absorbs maximally near 870 nm

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Here, chromatophore vesicles have been enriched with phospholipid by fusion to small unilamellar liposomes. Functionally intact fused preparations with increased phospholipid contents of >15-fold have been obtained and their fluorescence yield properties have permitted an assessment of the role of phospholipid associations in the arrangement of the light-harvesting system.

## 2. MATERIALS AND METHODS

Chromatophores with a photosynthetic unit size of ~80 mol BChl/mol P870 and a B800-850/B875 molar ratio of ~2.0 were essentially prepared as in [2] from *R. sphaeroides* NCIB8253 grown phototrophically at 30°C and 1800 lux.

Small unilamellar liposomes were prepared by sonication of suspensions of phosphatidylcholine type IV-S (Sigma, St Louis, MO) and fused to chromatophores as in [10] by lowering the pH to 6.0 to 6.2 in a buffer consisting of 1 mM Hepes, 10 mM sucrose, 30 mM mannitol, pH 7.4. Alternatively, chromatophores were fused with liposomes by the PEG-$CaCl_2$ procedure in [11] using 20 mM Tris-HCl buffer, pH 7.5. The fused membranes collected on a sucrose cushion and dialyzed overnight were subjected to sucrose density gradient centrifugation (fig.1). SDS-polyacrylamide gel electrophoresis was performed as in [2] on gels prepared with a gradient of 10–15% acrylamide. Membrane protein concentration was determined as in [12] and the procedures for BChl and phospholipid determinations were those in [13].

P870 activity was measured under saturating constant illumination on a Johnson Research Foundation DBS-3 spectrophotometer at 605–540 nm using the extinction coefficient in [14]. This instrument was also used for measurements of ~300 K fluorescence emission. Excitation was at 590 nm via a J-Y H20 600IR monochromator. Emission measured through 870- and 900-nm band-pass filters of 10-nm half-width (Omega Optical, Brattleboro, VT) was corrected for the response of the measuring system by comparison to a known chromatophore emission spectrum and for crossover of emission bands with spectra of homogeneous B800-850 and B875 complexes [2]. Low-temperature absorption, excitation and emission spectra were obtained with a single-beam spectrophotometer as in [4].

## 3. RESULTS AND DISCUSSION

When small-unilamellar liposomes and chromatophores were subjected to the PEG procedure, 4 distinct bands were observed after sucrose density gradient centrifugation (fig.1). Bands 4–1 sedimented between unfused chromatophores and free liposomes and contained increasing amounts of exogenous phospholipid (table 1). Three pigmented bands were observed with the acidic-pH procedure (not shown). Negatively stained PEG bands 4–1 appeared in electron micrographs as vesicles of increasing size essentially free from adsorbed liposomes [15]. Freeze-fracture studies following the acidic-pH [16] and PEG [17] procedures have established that true bilayer fusion occurs between liposomes and *R. sphaeroides* chromatophore membranes. Fractions similar to those obtained here were shown to consist of large unilamellar vesicles in which the

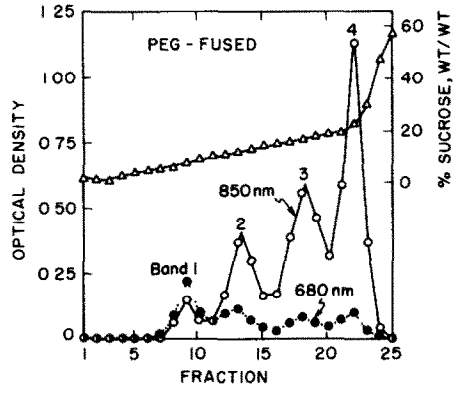


Fig.1. Isolation of lipid-enriched chromatophores. PEG-induced fusion was performed with small unilamellar liposomes as described in the text. The PEG-treated preparation (5.8 mg phospholipid plus chromatophores containing 100 $\mu$g BChl) was centrifuged on a discontinuous density gradient (prepared with 5, 10, 15 and 60% w/w sucrose) at 40000 rpm (284000 × $g_{av}$) and 4°C in a Beckman SW40Ti rotor for 4 h. 850 nm, in vivo absorption maximum of BChl; 680 nm, light scattering due mainly to liposomes. A mixture of chromatophores and liposomes subjected to the same centrifugation procedure gave only bands located just below band 4 and just above band 1, respectively.

Table 1

Composition and photochemical activities of bands from density gradient centrifugation

| | BChl/protein (nmol/mg) | Phospholipid/protein (w/w) | (-fold increase) | Reaction center (mmol P870/ mol BChl) | Carotenoid change[a] ($\Delta A$/nmol reaction center) |
|---|---|---|---|---|---|
| Chromatophores | 66.7 | 0.36 | – | 12.2 | 0.28 |
| Acidic-pH fusion | | | | | |
| Band 3 | 70.4 | 0.70 | 1.9 | 11.1 | 0.23 |
| Band 2 | 64.3 | 1.29 | 3.6 | 10.5 | 0.12 |
| Band 1 | 58.9 | 6.59 | 18.3 | 11.1 | 0.07 |
| PEG-fusion | | | | | |
| Band 4 | 47.6 | 0.57 | 1.6 | 11.9 | 0.08 |
| Band 3 | 61.4 | 2.41 | 6.7 | 13.0 | 0.13 |
| Band 2 | 51.9 | 5.58 | 15.5 | 12.5 | 0.11 |
| Band 1 | – | 25.5 | 71.0 | – | – |

[a] Obtained from absorption difference spectra under continuous illumination as described in the text. Measured in chromatophores from the maximum absorption change at 523–507 nm. These wavelength pairs were shifted to lower wavelengths in the fused preparations in which the zero point of the spectrum went from approx. 514 nm in chromatophores to about 509 nm in band 1 from the acidic-pH fusion procedure. The response in the presence of 2 $\mu$M valinomycin was subtracted from each spectrum

distance between randomly distributed intramembrane particles was significantly increased in comparison to chromatophores. Furthermore, it has been demonstrated recently [18] that the ubiquinone pool within the bilayer is diluted after fusion of chromatophores with liposomes by the acidic-pH procedure.

Absorption spectra at 77 K (fig.2) indicated that although the major light-harvesting BChl and carotenoid bands were retained, B800 losses occurred, especially after acidic-pH fusion. This was accompanied by the appearance of a new band at ~690 nm and a shoulder near 760 nm due to bacteriopheophytin formation. With the PEG-fusion procedure, some B850 was also lost (see fig.2 and table 1); nevertheless, the PEG-fused preparations retained significant B800 in comparison with acidic-pH fused membranes. In SDS-polyacrylamide gel electrophoresis, no major alterations in levels of reaction center or light-harvesting polypeptides were observed in the fused preparations (not shown).

Table 1 also shows that with each procedure, membrane fractions were obtained with various phospholipid enrichment up to >15-fold which retained significant BChl levels. P870 activities indicated that the reaction centers remained functionally intact. The ability to generate a membrane potential as measured from light-induced carotenoid absorption changes was reduced ~60–75% in highly phospholipid-enriched preparations. Since this response is confined to B800-850 carotenoids [20,21], the reduced amplitude of the change may reflect B800 losses [21] as well as changes in ion permeability, capacitance and charge. In this connection, charge would be delocalized over an increased membrane surface area in the fused preparations, resulting in generation of a weaker field. Overall, these results suggest that the fused membrane preparations have remained largely functionally intact.

The fluorescence yield properties of the B800-850 and B875 light-harvesting complexes were examined as probes of the structural organization and intercomplex associations within the phospholipid-enriched bilayers. Estimates at ~300 K indicated that with each fusion procedure, fluorescence emission from B850 relative to that from B875 was increased by about 2–3.5-fold in membrane preparations with significant phospholipid enrichment. Fig.3 shows this increased fluorescence emission from B850 at 77 K;

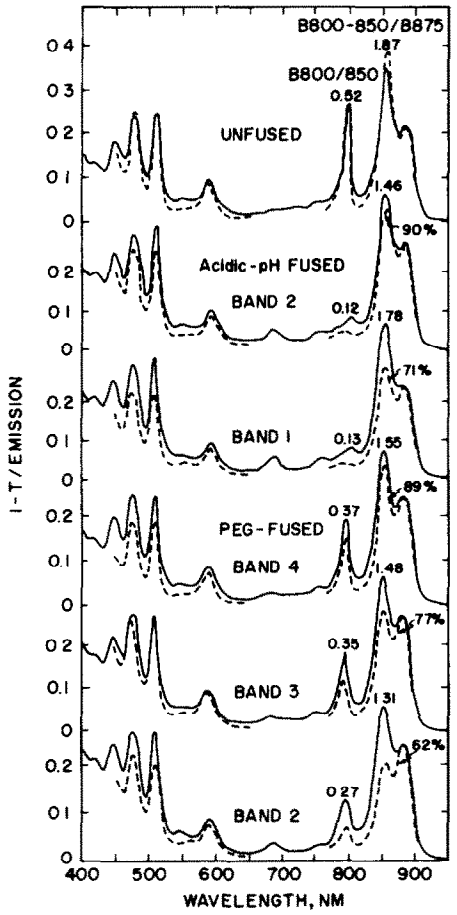


Fig.2. Fractional absorption (——) and fluorescence excitation (– – –) spectra of lipid-enriched membrane preparations at 77 K. Cuvettes (2.5-mm light path) contained 5 $\mu$g BChl/ml. Fractional absorption ($1 - T$) and 910-nm fluorescence emission (arbitrary units) were equalized at 885 nm, the absorption maximum of the B875 complex at 77 K. The spectra were corrected for light scattering by adjustment of the ratios of absorbance at 855 nm to that at 650 nm to 25:1. The molar ratios of B800/850 and B800-850/B875 are given for each preparation and were determined from 300 K absorption spectra with the extinction coefficients in [19] after computer-assisted correction for crossover of absorption bands. The efficiency of energy migration from B850 to B875 is also presented which was calculated from comparison of emission and fractional absorption at 855 and 885 nm.

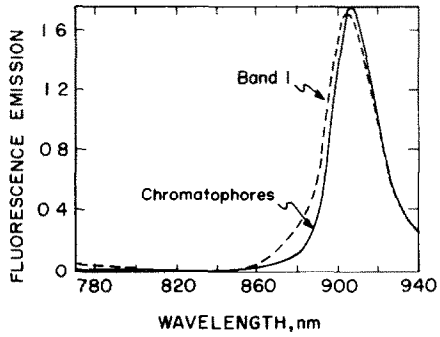


Fig.3. Near-infrared fluorescence emission spectra of chromatophore and acidid-pH fusion band 1 preparations at 77 K. Excitation was at 590 nm. Other conditions were those in fig.2. In chromatophores subjected to the acidic-pH and PEG procedures in the absence of liposomes, the respective fluorescence emission from B850 relative to that from B875 at ~300 K was 97.6 and 100.5% that of untreated controls. This was in contrast to the 2–3.5-fold increases in phospholipid-enriched preparations and indicated that the procedures alone caused no significant interruption in energy transfer between these antennae.

however, fluorescence quenching was observed in some of these phospholipid-enriched preparations. This may have resulted from phosphatidylcholine preparations used to prepare the liposomes, since when liposomes formed from native lipids were used, no apparent fluorescence quenching was observed [15]. In such fused preparations of large photosynthetic unit size, emission increases at ~300 K were confined to B800-850 and the slight decreases in emission from B875 suggested that the B875-reaction center cores remained intact [15].

Analysis of fluorescence excitation spectra at 77 K, also shown in fig.2, revealed that energy transfer efficiency from B850 to B875 was decreased to about 62 and 71% in the most phospholipid-rich preparations from the PEG and acidic-pH procedures, respectively (fig.4). Furthermore, a gradual reduction in the efficiency of this intercomplex energy migration was observed as phospholipid levels increased. In contrast, intracomplex energy migration was essentially unaffected as shown by much smaller decreases in efficiencies from carotenoids at 510 nm and from B800 in the PEG preparations (fig.4); the small decrease with carotenoids may have resulted from uncoupling of the B800 carotenoid pool [4] during the BChl 800 losses. Similar decreases in the efficiency of excitation energy transfer between B850 and B875 at 77 K were also observed in chromatophores fused by the acidic-pH procedure

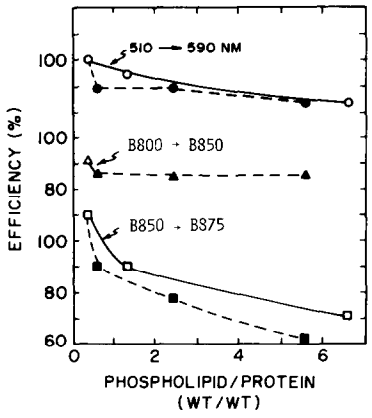


Fig.4. Efficiency of excitation energy transfer in lipid-enriched membrane preparations at 77 K. Calculated from excitation and fractional absorption spectra presented in fig.2. For calculation of the efficiency of energy migration from carotenoids at 510 nm to BChl, fluorescence emission elicited by excitation at 590 nm in the BChl-$Q_x$ band was equalized to the fractional absorption at this wavelength. The efficiency of energy migration in the 800 and 855 nm BChl-$Q_y$ bands was calculated after equalizing at 885 nm as shown in fig.2. Open symbols, unfused and acidic-pH fused membrane preparations; closed symbols, PEG-fused membrane preparations. No significant decreases in efficiency of excitation energy transfer were observed in these absorption bands at ~300 K in chromatophores subjected to the acidic-pH and PEG procedures in the absence of liposomes.

to liposomes prepared with native lipid (Den Ouden, A., Van Dorssen, R.J., Pennoyer, J.D., Amesz, J. and Niederman, R.A., unpublished). Thus, the observed results do not depend upon specificity within the commercial lipid preparations.

Overall, these results suggest that added lipid alters the organization of the membrane such that part of the B800-850 antenna becomes structurally and functionally detached from the rest of the photosynthetic unit and diffuses over the increased bilayer surface. In this connection, detachment of chlorophyll-protein complex II antenna was demonstrated recently in spinach thylakoid membranes fused with liposomes [22]. It is possible that the selective dissociation observed here may be confined to B800-850 domains at the periphery of photosynthetic units and that these B800-850 phospholipid interactions normally assist in maintaining organizational stability within the light-harvesting system.

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