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PHOSPHOLIPID VESICLE FUSION MONITORED BY FLUORESCENCE ENERGY TRANSFER

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SUMMARY

A method is presented which allows the observation of phospholipid vesicle fusion by the occurrence of Förster resonance energy transfer between the amphiphilic probes dansyldipalmitoylphosphatidylethanolamine and 3-[4-(p-N,N-didecylaminostyryl)-1-pyridinium]-propylsulfonate. This method is applied to the Ca^{++} mediated fusion of phosphatidyl serine vesicles.

INTRODUCTION

Fluorescent probes are among the most important tools in membrane research but have been only occasionally applied to studies of membrane fusion (1-4). The methodology outlined in this work provides a simple procedure for studying phospholipid vesicle fusion by monitoring the extent of resonance energy transfer between unlike probes. The success of this procedure should provide the basis for extension of the methodology to the study of long-range interactions between the membranes of vesicles and cells (5).

MATERIALS AND METHODS

Dansyl-dipalmitoyl phosphatidyl ethanolamine was prepared as previously described (6); p-(N,N-didecylamino)styrylpyridinium propylsulfonate (DI-10-ASPPS)[2] was the gift of Dr. H. Parkins of this department. Bovine brain phosphatidylserine was purchased from Sigma Biochemicals and egg phosphatidylcholine was obtained from Calbiochem. Both were used as obtained. The buffer system was 50 mM tris (Gold Label, Aldrich Chemical Co.) which was adjusted to pH 7 by addition of HCl in double distilled water.

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[2] Abbreviations used in this work: DI-10-ASPPS = 3-[4-(p-N,N-didecylaminostyryl)-1-pyridinium]-propylsulfonate; R.E.T. = resonance energy transfer; DDPPE = dansyldipalmitoylphosphatidylethanolamine; PS = phosphatidyl serine.

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Vesicles were prepared as follows. Solutions containing the lipid and dyes were evaporated in a cylindrical vial under a stream of nitrogen. Buffer was added to the resulting film and the sample was sonicated for 15 min at 0° under a stream of N_2. Sonication was done at 30% power using a Branson Model # W-185 sonicator. All spectrofluorometric measurements were done using a Perkin-Elmer MPF-44B spectrofluorometer interfaced to a Bascom-Turner Model # 8110 recorder. This recorder has data storage and processing capabilities which allowed generation of the difference spectra displayed in this work. Kinetic runs using phosphatidylserine vesicles were done in a thermostatted cuvette holder at 39°C.

DISCUSSION AND RESULTS

Briefly, the experimental design involves the establishment of two populations of vesicles, one containing a membrane bound fluorescent donor probe and one containing a similarly bound acceptor probe. The probes are chosen according to two criteria, the donor and acceptor probes must interact via a Förster R.E.T. mechanism (7), and the probes must bind tightly to the membrane with dissociation kinetics that are slow on the timescale of the fusion process.

One such system of probes uses DDPPE as the donor probe and DI-10-ASPPS as the acceptor probe. Evaluation of Förster equation using the appropriate spectral parameters for this probe pair gives a critical transfer distance (R_0) of 36 Å; so this pair satisfies the first criterion.

Satisfaction of the binding criterion for DI-10-ASPPS was explicitly demonstrated with egg lecithin vesicles at room temperature. A small volume of concentrated ethanolic solution of DI-10-ASPPS (final concentration of 5×10^{-6} M) was injected into a 2 mg/ml egg lecithin dispersion and the fluorescence spectrum was observed as a function of time. Since aqueous DI-10-ASPPS has a much smaller fluorescence quantum yield than membrane bound dye we observe a steady increase in fluorescence intensity with a half life of 10 hr. We felt that derivitization of phosphatidylethanolamine would not alter its strong binding to the membrane sufficiently to allow rapid equilibration of bound and free probe. This was borne out in the specific experimental system that we used in this work.

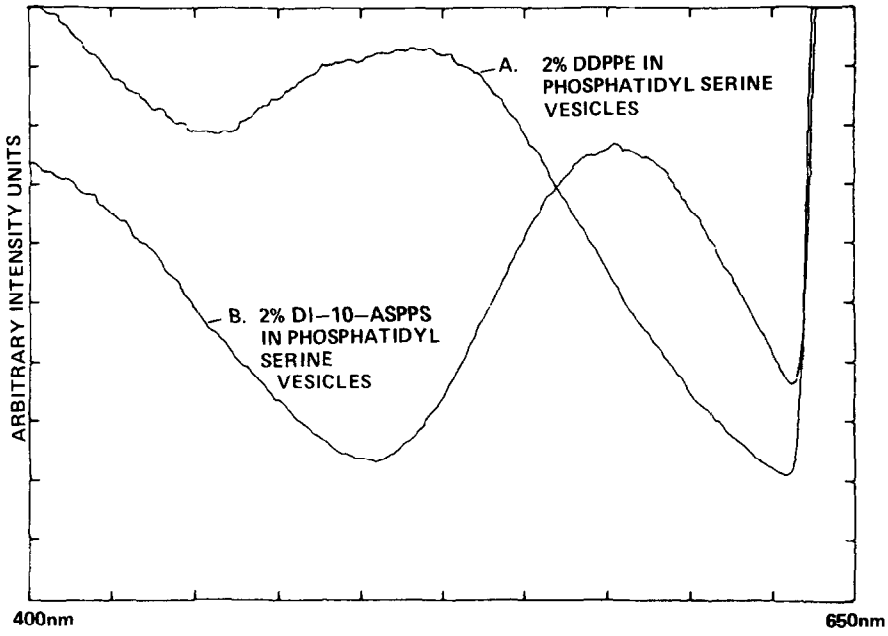

Figure 1. Emission spectra λ_E = 325 nm [PS] = 0.01 mg/ml. A sharp increase in intensity is observed as we approach the excitation wavelength in the second order of the monochromator. This results in occasional distortion in spectra (Figures 2,3,4) in this region.

We have chosen to perform our initial fusion studies on the well described phosphatidyl serine (PS) system. Several investigators (8,9) have shown that PS vesicles, usually stable for long periods, rapidly fuse in the presence of Ca^{++}. Two PS (0.01 mg/ml) vesicle suspensions were prepared in 50 mM tris·HCl buffer, one (A) containing 2% DDPPE, and one (B) containing 2% DI-10-ASPPS (see Figure 1). A mixture of equal volumes of these suspensions should give an emission spectrum identical to $\frac{1}{2}$ spectrum A + $\frac{1}{2}$ spectrum B, if no fusion or diffusive mixing of the probes occurs. Comparing spectra so obtained (Figure 2) we can see no evidence of Förster R.E.T. behavior. At 39°C this mixture will remain stable for over 10 hr. If we make vesicles which contain both 1% DI-10-ASPPS and 1% DDPPE by co-sonication and compare the emission spectrum obtained from this dispersion with the sum of individual spectra, we see (Figure 3) clear evidence of R.E.T. in both the donor (515 nm) and acceptor (600 nm) fluorescence.

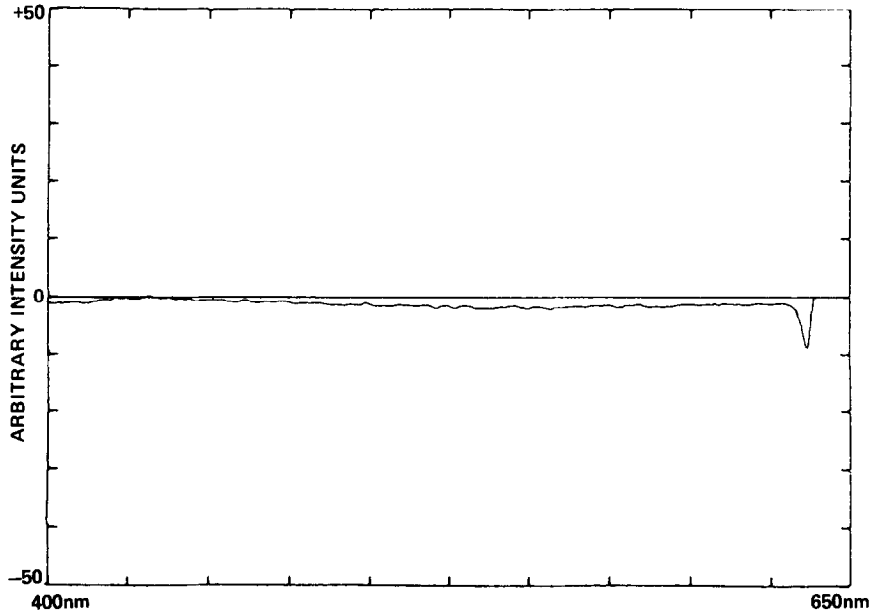

Figure 2. Difference between an emission spectrum of a mixture of separately labeled donor and acceptor containing vesicles and 1/2 of the sum of spectrum A and spectrum B in Figure 1.

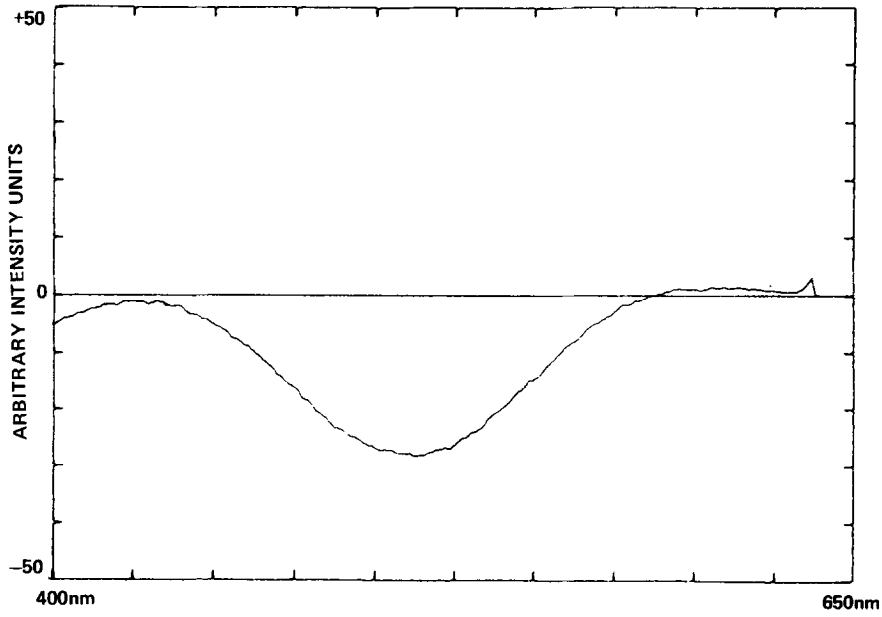

Figure 3. Difference between an emission spectrum of vesicles containing 1% DDPPE and 1% DI-10-ASPPS, and 1/2 of the sum of spectrum A and spectrum B in Figure 1.

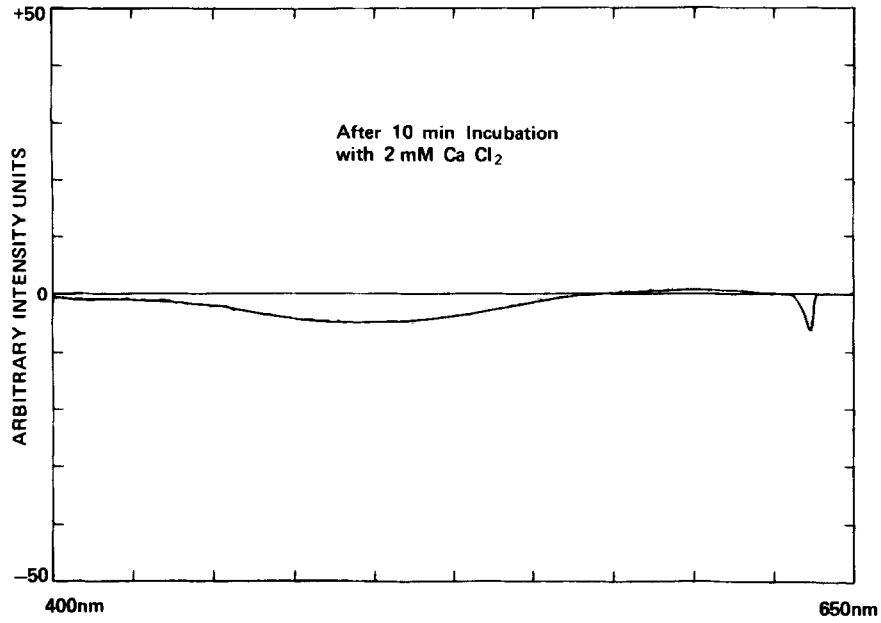

Figure 4. The emission spectrum of a mixture of separately labeled donor and acceptor containing vesicles incubated with 2 mM Ca^{++} was obtained and stored. Subtracted from this was 1/2 of the sum of the spectra of the individually labeled vesicles which had also been treated with Ca^{++}. The above difference spectrum clearly shows fluorescence energy transfer.

When donor containing and acceptor containing vesicles are caused to fuse in the presence of 2 mM $CaCl_2$ mixing of the surface lipids is demonstrated by the appearance of R.E.T. (Figure 4). The magnitude of the effect is diminished compared to Figure 3 since only part of the vesicle fusions will occur between vesicles which contain different probes.

In summary, Ca^{++} induced fusion is shown to proceed with concomitant appearance of Förster resonance energy transfer between DDPPE and DI-10-ASPPS. It is further shown that this behavior does not occur in the absence of fusion (i.e., by passive diffusion of the probes). We plan to extend our study of this system to faster timescales to help resolve some of the recently raised (9) questions regarding the mechanism of PS vesicle fusion. The potential of this general method for the study of vesicle-vesicle and cell-cell aggregation is described in the accompanying paper.

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