Anomalous Dependence of Pyrene Spectra and Lifetimes with Temperature in Large Unilamellar Vesicles from Dioctadecyldimethylammonium Chloride and Dipalmitoylphosphatidylcholine

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Lifetimes of pyrene incorporated into large unilamellar vesicles of dipalmitoylphosphatidylcholine and dioctadecyldimethylammonium chloride increase when the temperature increases prior to the melting temperature of the bilayers. These changes are paralleled by a decrease in the vibronic bands intensity ratio I_I/I_{III} of the fluorescence spectra, indicative of water expulsion and/or a deeper penetration of the probe prior to the bilayer phase transition. In DPPC vesicles, both pyrene lifetimes and the I_I/I_{III} ratios show at the phase transition temperature changes that are indicative of increased water penetration.

Introduction

In two recent works, Weiss et al.[1,2] reported on a peculiar behavior displayed by singlet lifetimes of pyrene and pyrene derivatives when employed as reporters of local microenvironments and thermotropic phase transitions in various microstructures from phospholipid and surfactant bilayers. The peculiar behavior observed is related with an unexpected increase of the probe's lifetime as temperature is raised in the vicinty of the phase transition region of the microstructure considered. The effect has been observed in small unilamellar vesicles (SUV's) and tubule phases formed by phospholipids[1] and in the gel and middle phases of stearate surfactants.[2] The phenomenon was also observed in a study of the quenching of pyrene singlets by amines in liquid crystalline solvents.[3] These reports prompted us to communicate on closely related results that we have obtained in large unilamellar vesicles (LUV's) formed by a synthetic surfactant, dioctadecyldimethylammonium chloride (DODAC), and by dipalmitoylphosphatidylcholine (DPPC).

Experimental Section

DPPC (Avanti) was used as received. DODAC was a sample kindly supplied by Professor H. Chaimovich (U. de Sao Paulo), purified as described previously.[4] Pyrene (Py), [11-(1-pyrenyl)-undecyl]trimethylammonium iodide (PUTM), 16-(1-pyrenyl)-hexadecanoic acid (PH), and 9-(1-pyrenyl)nonanoic acid (PN) from Molecular Probes were employed without treatment. LUV's were prepared following the injection with simultaneous vaporization of the solvent (chloroform) method.[5,6] The experimental arrangement for injection was similar to that described by Deamer and Baugham.[6] DPPC vesicles were prepared in Tris-HCl (pH = 7.4) buffer; DODAC vesicles were prepared in water. PUTM in DPPC and PH and PN in DODAC were incorporated into the vesicles by adding the probes to a chloroform solution prior to injection. Py was incorporated either by following the same procedure or by adding a small aliquot of a concentrated solution in methanol to the already prepared vesicle solutions. When the latter procedure was employed, the samples were incubated for ca. 30 min at 60 °C, a temperature above the vesicle's transition temperature. [Probe]/ [DPPC] or [DODAC] mole ratios employed were below 10^{-4}. Experiments were performed at 1 mg/mL and 2 mM concentrations for DPPC and DODAC, respectively. When Py was employed as probe, almost total incorporation into the vesicles was assessed by the lack of quenching by acrylamide. Addition of acrylamide (60 mM) almost totally quenches the emission from Py in the buffer solution. On the other hand, in the presence of vesicles, the same concentration of acrylamide decreases Py emission by less than 5%.

Fluorescence spectra were recorded on a Perkin-Elmer LS5 luminescence spectrometer. Lifetimes were measured by following the fluorescence decay after excitation with a Nitromite LN100 nitrogen laser.

Results and Discussion

Pyrene and the pyrene derivatives employed show, at the low probe to surfactant ratios employed (probe/surfactant mole ratio < 10^{-4}), only monomeric emission. Fluorescence intensity decays were well fitted to a monoexponential function in all cases but for DPPC vesicles far below the phase transition temperature. In this later case, the lifetime values reported correspond to those obtained by treating as monoexponential the fluorescence intensities measured from ca. 40 ns after excitation.

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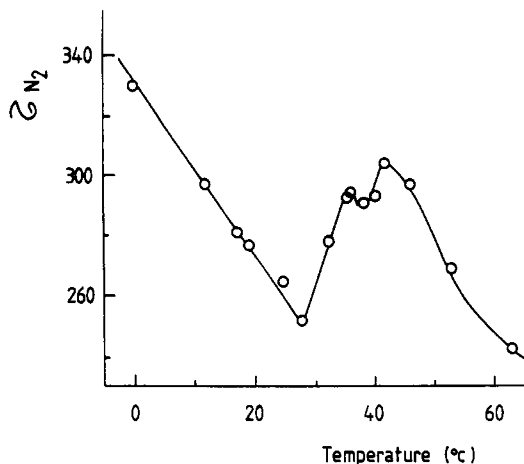

Figure 1. Pyrene lifetimes (nanoseconds) in DPPC vesicles as a function of temperature (concentration of DPPC, 1 mg/mL).

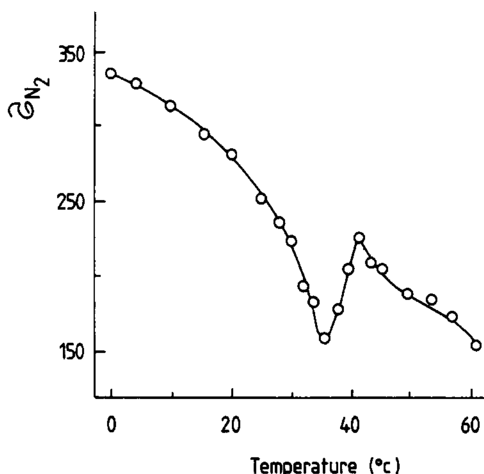

Figure 2. Pyrene lifetimes (nanoseconds) in DODAC vesicles as a function of temperature (DODAC concentration, 2 mM).

Figures 1 and 2 show the results obtained for the dependence of the pyrene lifetimes (in nitrogen-purged solution) with temperature in DPPC and DODAC LUV's. The behavior observed closely parallels that reported by Weiss et al. in SUV's and tubules from phospholipids[1] and in the gel and middle phases of stearate surfactants;[2] i.e., after decreasing with temperature, the Py lifetimes show a sudden increase when the temperature reaches the region of the vesicle's phase transition. However, and contrary to the effect previously reported,[1,2] probes that can be considered to be deeply located into the lipid bilayer (i.e., PUTM, PH, and PN) show a very limited increase in lifetime over the same temperature range (see Figure 3 for PH data in DODAC vesicles). Similar results were obtained for PUTM in DPPC and PN in DODAC vesicles. In these systems, the lifetimes under nitrogen increase from 203 to 208 ns (PUTM in DPPC) and from 159 to 180 ns (PN in DODAC) in the vicinity of the vesicle's phase transition temperature. The difference between the present results and those previously reported can be rationalized in terms of the dependence of the observed phenomenon on assembly order.[2]

The observed changes in pyrene lifetimes can be due to water expulsion and a deeper incorporation of pyrene into the bilayer in the temperature zone prior to the phase transition, putting it away from the aqueous interfacial zone. This is supported by the reported changes in the vibronic band intensity ratio, I_I/I_{III}, of pyrene fluorescence spectra in large DODAC vesicles[7] and small, sonicated, DPPC vesicles.[8] A similar effect is noted in the large DPPC

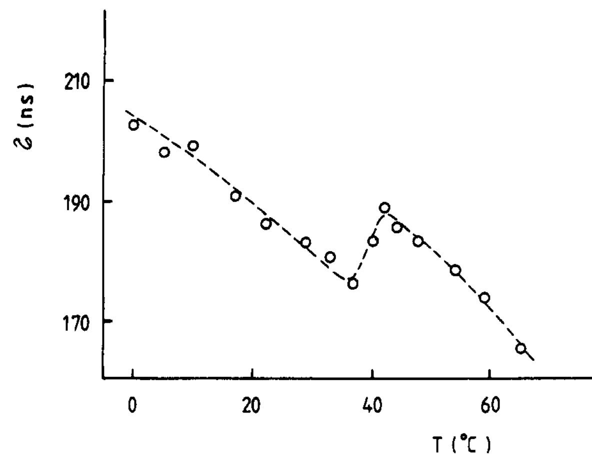

Figure 3. 16-(1-Pyrenyl)hexadecanoic acid lifetimes in DODAC vesicles as a function of temperature (DODAC concentration, 5 mM).

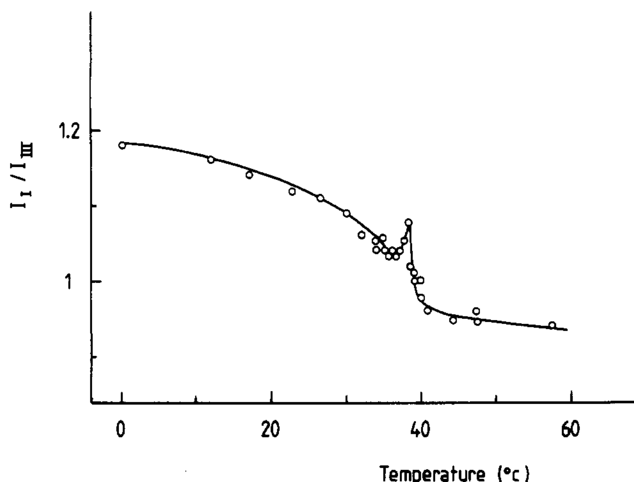

Figure 4. Changes in the vibronic band intensity ratio, I_I/I_{III}, of pyrene fluorescence spectra as a function of temperature in DPPC vesicles.

vesicles (See Figure 4) where a considerable decrease in the I_I/I_{III} ratio occurs in the 25–43 °C temperature range, the same as over which a sharp increase in pyrene lifetime is observed.[9] It is interesting to point that all these changes take place prior to the melting of the vesicles. This is particularly so for DPPC, that neatly fuses at 41.5 °C,[10] while the increase in pyrene lifetime takes place over a 17 °C range. In DPPC vesicles at temperatures below the pretransition, the fluorescence decays were poorly fitted to a monoexponential function, indicating that the pyrene ensemble does not constitute an homogeneous population. This could indicate the presence of pyrene molecules located at different sites that do not exchange during the lifetimes of the excited molecules and/or the partial association of the pyrene molecules to the surfactant head.[8,11,12] In DODAC vesicles and in DPPC at higher temperatures, the decays are very well fitted to monoexponential functions, showing that the probes can be considered to constitute a homogeneous population. In

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DODAC vesicles this is further supported by the invariance of the I_I/I_{III} ratio on the extent of quenching by iodide ions, either when these counterions are bound exclusively to the outer interface or also when they are incorporated to the vesicular pool.[13]

S-shaped changes in DPPC bilayers over a similar temperature range have also been observed when the excimer to monomer fluorescence ratio of pyrene was measured as a function of temperature[8,14] or lysophosphatidylcholine addition.[14] In these works, the sharp decrease in excimer formation prior to the phase transition was also assigned to penetration of pyrene into the bilayer in the pretransition temperature range. Also, an S-shaped dependence of pyrene lifetime under nitrogen was observed in small DPPC vesicles upon lysophosphatidylcholine addition and the results were explained in terms of water penetration at low additive incorporation followed by mixed micelles formation as a consequence of the bilayer break up.

Pyrene lifetimes (Figure 1) and the shape of the fluorescence spectra (Figure 4) in DPPC vesicles, when plotted as a function of temperature, show a small peak in the vicinity of the phase transition temperature.[10] These changes can be interpreted in terms of a particularly high water penetration at the phase transition temperature, leading to a decrease in pyrene lifetime and an increase in the I_I/I_{III} ratio.[9] The peculiar profiles observed, particularly in DPPC, can be explained by a deeper pyrene penetration into the bilayer *prior to* the melting temperature plus a significant water penetration *at* the melting temperature.

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