

OXYGEN QUENCHING OF PYRENE-LIPID FLUORESCENCE IN PHOSPHATIDYLCHOLINE VESICLES

A Probe for Membrane Organization

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ABSTRACT Oxygen quenching has been used as an alternative method to study the temperature dependence of the apparent excimer formation constant, k_{dm} , of *N*-(10-[1-pyrene]-decanoyl)-sphingomyelin (Pyr-SPM) in 1-palmitoyl-2-oleoyl-L- α -phosphatidylcholine (POPC) multilamellar vesicles. In conjunction with the lifetime of Pyr-SPM monomer in the absence of excimer and oxygen, k_{dm} can be determined from the measurements of the monomer intensity as a function of oxygen concentration. The advantage of this method is that k_{dm} can be determined without knowledge of the excimer lifetime and intensity, and without knowledge of the true concentration of oxygen in lipid bilayers. Our results show that k_{dm} increases monotonically with temperature from 16 to 40°C, becomes insensitive to temperature from 40 to 50°C and increases again at 54°C. The temperature-insensitive region corresponds to the temperature range of the phase transition of Pyr-SPM determined by differential scanning calorimetry. This result suggests the existence of Pyr-SPM-enriched domains in POPC vesicles. In contrast, no abrupt change in k_{dm} with temperature occurs in the case of 1-palmitoyl-2-[10-(1-pyrenyl) decanoyl] phosphatidylcholine (Pyr-PC).

INTRODUCTION

The fluorescence of pyrene or pyrene derivatives has been used extensively in membrane studies (for example, see Galla and Sackmann, 1974; Vanderkooi and Callis, 1974; Hartmann and Galla, 1978; Galla et al., 1979; Massey et al., 1982). Among these studies, the most frequently used method has been the measurement of the ratio of excimer intensity, I_d , to monomer intensity, I_m , as a function of temperature or as a function of concentration of the pyrene moiety. I_d/I_m is useful because it permits the determination of the excimer formation constant, k_{dm} , by Eq. 1 (Birks et al., 1963)

$$\frac{I_d}{I_m} = \frac{k_{fd}}{k_{fm}} k_{dm} Y^{-1} c. \quad (1)$$

Here c is the pyrene concentration, k_{fd} is the emission rate of excimer fluorescence, k_{fm} is the emission rate of monomer fluorescence, and Y^{-1} is the lifetime of excimer. Under certain conditions (Forster and Kasper, 1955; Birks et al., 1963; Birks et al., 1970; Vanderkooi and Callis, 1974; Galla and Sackmann, 1974; Andre et al., 1979), k_{dm} can be directly related to the diffusion coefficient of the pyrene moiety in the medium by the following equation (Smoluchowski, 1917)

$$k_{dm} = 4\pi\sigma_{mm} N(D_m + D_m^*)/1,000, \quad (2)$$

where D_m and D_m^* are the diffusion coefficients of ground

state monomer, M , and excited state monomer, M^* , respectively; N , Avogadro's number; σ_{mm} , the sum of the molecular radii of M and M^* . Therefore, if pyrene or its derivatives are incorporated into bilayer membranes, the value of k_{dm} should yield information regarding the immediate environment of the pyrene moiety. In this sense, k_{dm} is a measure of the microscopic diffusion constant of the lipid component. In contrast, the diffusion coefficient determined by the method of fluorescence recovery after photobleaching (FRAP) provides a measure of a macroscopically defined lateral diffusion coefficient.

Although Eq. 1 can be used to determine k_{dm} , the precise determinations of k_{fd} , k_{fm} , and Y^{-1} are not generally easy, and consequently the resulting k_{dm} may have considerable errors. Instead, in this study, we have determined k_{dm} by a new method based on measurement of the effect of excimer formation on the quenching of the fluorescence of the monomer of pyrene lipid by molecular oxygen. The theory is described in the Appendix. The advantage of this method is that k_{dm} can be determined without knowledge of excimer lifetime and intensity, and without knowledge of the local concentration of oxygen near the pyrene moiety. Moreover, the new method also provides information about the oxygen behavior in lipid bilayers. Such information should be useful for the studies of oxygen partition in lipid bilayers (Subczynski and Hyde, 1983) and oxygen quenching of fluorescence of lipoproteins in lipid bilayers (Mantulin et al., 1982).

Here the usefulness of this method is illustrated by a study of *N*-(10-[1-pyrene]-decanoyl)-sphingomyelin (Pyr-SPM) in 1-palmitoyl-2-oleoyl-L- α -phosphatidylcholine (POPC) vesicles and by a study of 1-palmitoyl-2-[10-(1-pyrenyl)decanoyl] phosphatidylcholine (Pyr-PC) in POPC vesicles. The primary purpose of this experimental work is to examine the Pyr-SPM/POPC system for evidence of compositional domain formation. It has been suggested that compositionally different domains may exist in binary lipid mixtures that are essentially liquid crystalline (Phillips et al., 1970; Shimshick and McConnell, 1973; Mabrey and Sturtevant, 1976; van Dijck et al., 1978; Correa-Freire et al., 1979; Klausner and Kleinfeld, 1984). Pyr/SPM at low concentrations in phosphatidylcholine vesicles may also be such a system. Recently Frank et al. (1983) have demonstrated that the temperature dependence of the spontaneous transfer rate of Pyr-SPM at low concentrations from POPC donor vesicles to POPC acceptor vesicles abruptly changes at $\sim 30^\circ\text{C}$ although POPC, the principal component, remains liquid crystalline over the whole temperature range. They have suggested on the basis of this observation that a gel phase rich in Pyr-SPM exists in POPC vesicles below $\sim 30^\circ\text{C}$. In an effort to obtain additional independent evidence of phase separation, we have examined the temperature dependence of k_{dm} of Pyr-SPM in POPC vesicles by the oxygen quenching method. We have in fact found an abrupt change in k_{dm} at a temperature range ($45 \pm 5^\circ\text{C}$) corresponding to the temperature range of the phase transition of Pyr-SPM, but not POPC, as determined by differential scanning calorimetry. In contrast, no abrupt change with temperature in k_{dm} is observed in the system composed of Pyr-PC in POPC vesicles, a system presumed to be liquid crystalline over the temperature range examined. This observation supports the suggestion that a Pyr-SPM enriched domain exists in POPC vesicles below this temperature range. A preliminary report of this work has appeared elsewhere (Chong and Thompson, 1984).

MATERIALS AND METHODS

Materials

Pyr-SPM was synthesized in our laboratory (Hresko, R. C., and T. E. Thompson, unpublished results). Pyr-PC was purchased from KSV-Chemicals Oy (Kauniainen, Finland). POPC was obtained from Avanti Biochemicals (Birmingham, AL). The oxygen/nitrogen gas mixtures were purchased from and analyzed by Matheson (Matheson Gas Products, Inc., Seacaucus, NJ).

Preparation of Vesicles

Pyr-PC (or Pyr-SPM) and POPC were first dissolved and thoroughly mixed in chloroform. After removing the organic solvent by evaporation, multilamellar vesicles of the mixed lipids were prepared in 50 mM KCl-0.02% NaN_3 by the method of Bangham et al. (1967).

Concentrations of POPC, Pyr-PC, and Pyr-SPM were determined as inorganic phosphate following Bartlett (1959). The concentration of Pyr-SPM or Pyr-PC was determined by measuring the OD at 334 nm

using an extinction coefficient of $50,000 \text{ M}^{-1} \text{ cm}^{-1}$ in methanol (Pownall and Smith, 1973).

Fluorescence Intensity Measurements

Fluorescence intensity measurements were made with an SLM spectrofluorometer (4800S; SLM Instruments, Inc., Urbana, IL). For measurement of monomer, the excitation light monochromator was set at 346 nm and the emission light monochromator was at 378 nm. The absorbance of the solution was <0.07 at 346 nm. To correct for scattered light in samples of multilamellar vesicles, identical intensity measurements were made on pure POPC multilamellar vesicle dispersions. Scattered light was $<1.5\%$ of the total measured emission of Pyr-SPM or Pyr-PC monomer. The excitation bandpass was set at 1 nm to minimize photobleaching. The precision of the intensity measurements was better than $\pm 0.2\%$.

The cuvette temperature was controlled to $\pm 0.2^\circ\text{C}$ by a thermostated circulator (RTE-8; Neslab Instruments, Inc., Portsmouth, NH) and monitored by a digital readout from a platinum resistance thermometer situated in a cuvette located in the same holder as the sample.

Fluorescence Lifetimes Measurements

Fluorescence lifetimes were measured on the SLM 4800S phase-modulation fluorometer (SLM Instruments, Inc.). Fluorescence lifetimes of Pyr-SPM or Pyr-PC were measured with respect to light scattered from a glycogen dispersion in water. For this measurement, the following conditions were used: excitation wavelength, 346 nm; emission wavelength, 346 nm for glycogen, 378 nm for the monomer of Pyr-SPM or Pyr-PC; light modulation frequency, 6 MHz. The effect of color delay (Rayner et al., 1976) between 346 nm (excitation) and 378 nm (emission) from the photomultiplier tube (9813 QA; Thorn EMI Ltd., Middlesex, England) was determined by the method of Jameson and Weber (1981). Since a $\Delta\tau_{\text{el}}$ (the time delay, as defined by Jameson and Weber) of only 91 ps was found, the lifetime of pyrene lipid was affected by the color delay by no more than 100 ps, which was less than the precision of the lifetime measurements (see Table II for examples). Thus corrections for this effect were not necessary. The temperature in the sample cuvette was controlled and determined as described above.

To calculate k_{dm} using Eq. 11, it is necessary to know the monomer lifetime in the absence of oxygen and in the absence of excimer, $\tau_{0\%}^0$. Oxygen can be eliminated by flushing the sample with an inert gas. Although lowering the mole percent of pyrene moiety can, in principle, reduce the excimer concentration to essentially zero, in practice it is impractical to do so. The difficulty is due to the fact that excimer emission becomes very weak when the level of pyrene derivative is small. Hence, to obtain $\tau_{0\%}^0$ we measured the lifetimes of Pyr-SPM (or Pyr-PC) in the absence of oxygen as a function of mole percent pyrene moiety. A value of $\tau_{0\%}^0$ can be obtained by extrapolation from a plot of $1/\tau_{0\%}$ vs. Pyr-SPM or Pyr-PC at a given temperature.

Preparation of Samples for Fluorescence Measurements

The fluorescence measurement used a special cuvette made from a long narrow glass tube fused to a regular 1 cm \times 1 cm quartz cuvette. The small opening of the cuvette was covered with a rubber septum, which itself was fitted into a special adaptor. A needle inserted through the adaptor and septum allowed addition of gas. Sealing the cuvette was accomplished by an on-off valve (model CV2; Laboratory Data Control, Riviera, FL) connected to the adaptor. The outlet of the on-off valve was immersed in a beaker of water such that the gas flow could be directly visualized. No appreciable gas leakage occurred during a 5 h period at temperatures between 5 and 55°C for samples sealed in accordance with this procedure, as evidenced by a constant pyrene-lipid lifetime over this period. Several different gases were used: 100% N_2 , 100% O_2 , 75:25% O_2/N_2 , 50:50% O_2/N_2 , 20:80% O_2/N_2 (air). Except in the case of 20:80%

O₂/N₂, lipid dispersions (1.5–2.0 ml) were equilibrated with each gas mixture by bubbling that gas through the solution for 5–10 min followed by flushing of the gas phase for 80 min. Prolonged flushing had no additional effect on the lifetime. Immediately following the removal of the needle, the on-off valve was closed at the moment when the last gas bubble was seen from the outlet of the valve. In so doing, the gas pressure inside the sealed cuvette was essentially atmospheric.

RESULTS

Fluorescence Intensity of Pyr-SPM Monomer and Pyr-PC Monomer

Emission spectra of Pyr-SPM in POPC vesicles at various oxygen concentrations are shown in Fig. 1. It is obvious that the quencher, oxygen, does not alter the emission maximum. This observation has previously been noted by Fischkoff and Vanderkooi (1975). Thus, the fluorescence intensity and lifetime of the monomer of pyrene-lipid can be monitored at 378 nm under all conditions.

The monomer fluorescence intensities were measured as a function of apparent oxygen concentrations, [O₂], or more precisely, the partial pressure of oxygen in the cuvette at 1 atm. The plot of F_m^0/F_m vs. [O₂] in the case of 0.84 mol % Pyr-SPM is given in Fig. 2 *a*. Appreciable quenching is evident.

F_m^0/F_m appears to be a linear function of apparent oxygen concentration. This linearity persists not only in the case of 0.84 mol % Pyr-SPM (Fig. 2 *a*) but also in the case of 4.07 mol % Pyr-SPM (data not shown). The slope of the

F_m^0/F_m vs. [O₂] plot was determined by a linear regression program. For each given temperature and each given mole percent Pyr-SPM, several experimentally determined slopes were obtained and averaged. The averaged slopes (see Table I) were used for the calculation of k_{dm} .

In the case of Pyr-PC in POPC vesicles, the plot of F_m^0/F_m vs. [O₂] is more complex. As shown in Fig. 2 *b*, upward curvature is obtained in the case of 0.9 mol % Pyr-PC in POPC. In general, the deviation from linearity becomes more pronounced at higher temperatures and at lower mole percent of pyrene-lipid. In these cases, initial slopes were used for the calculation of k_{dm} .

Fluorescence Lifetimes of Pyr-SPM Monomer and Pyr-PC Monomer

Examples of the fluorescence lifetimes of pyrene-lipid in POPC multilamellar vesicles in the absence of oxygen are given in Table II. It is noted that the phase-measured lifetimes, $\tau(\phi)$, are lower than the modulation-measured lifetimes, $\tau(M)$.

The plots of $1/\tau_{0\%}(M)$ vs. mole percent Pyr-SPM at different temperatures are shown in Fig. 3 *a*. At a given temperature, the data fall on a straight line. Similar linearity in the plot of $1/\tau$ vs. concentration of pyrene moiety has been reported previously by others (Donner et al., 1980) in their studies on free pyrene molecules. The reciprocal of the y -intercept in Fig. 3 is the modulation-

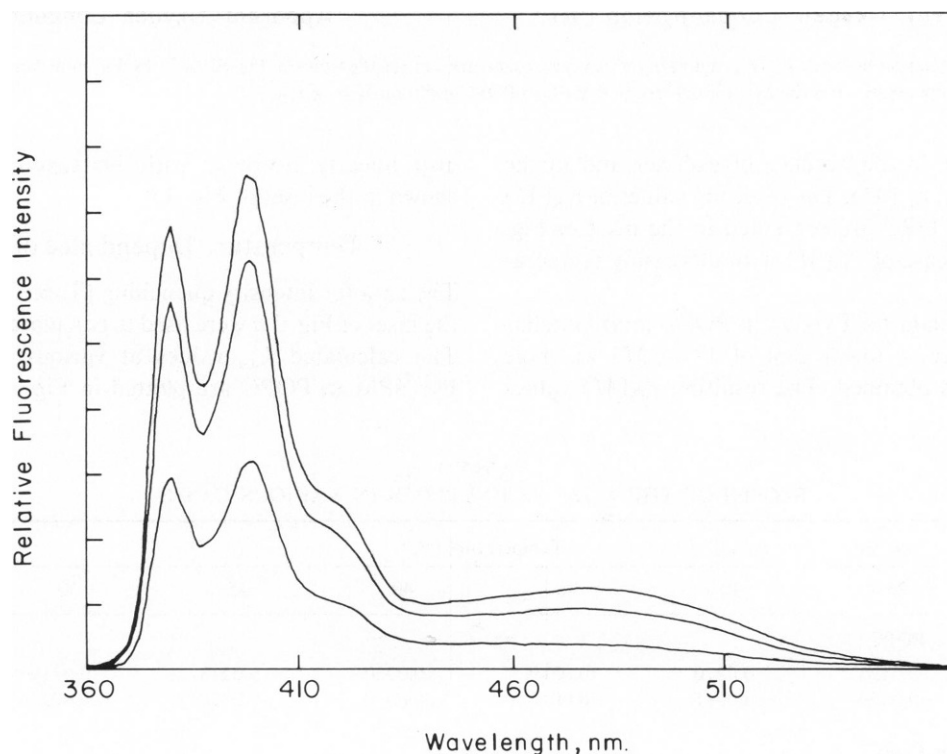


FIGURE 1 Emission spectra of 2 mol % Pyr-SPM in POPC vesicles under nitrogen (*top*), 20:80% O₂/N₂ (*middle*), and 100% O₂ (*bottom*). Temperature was 20°C.

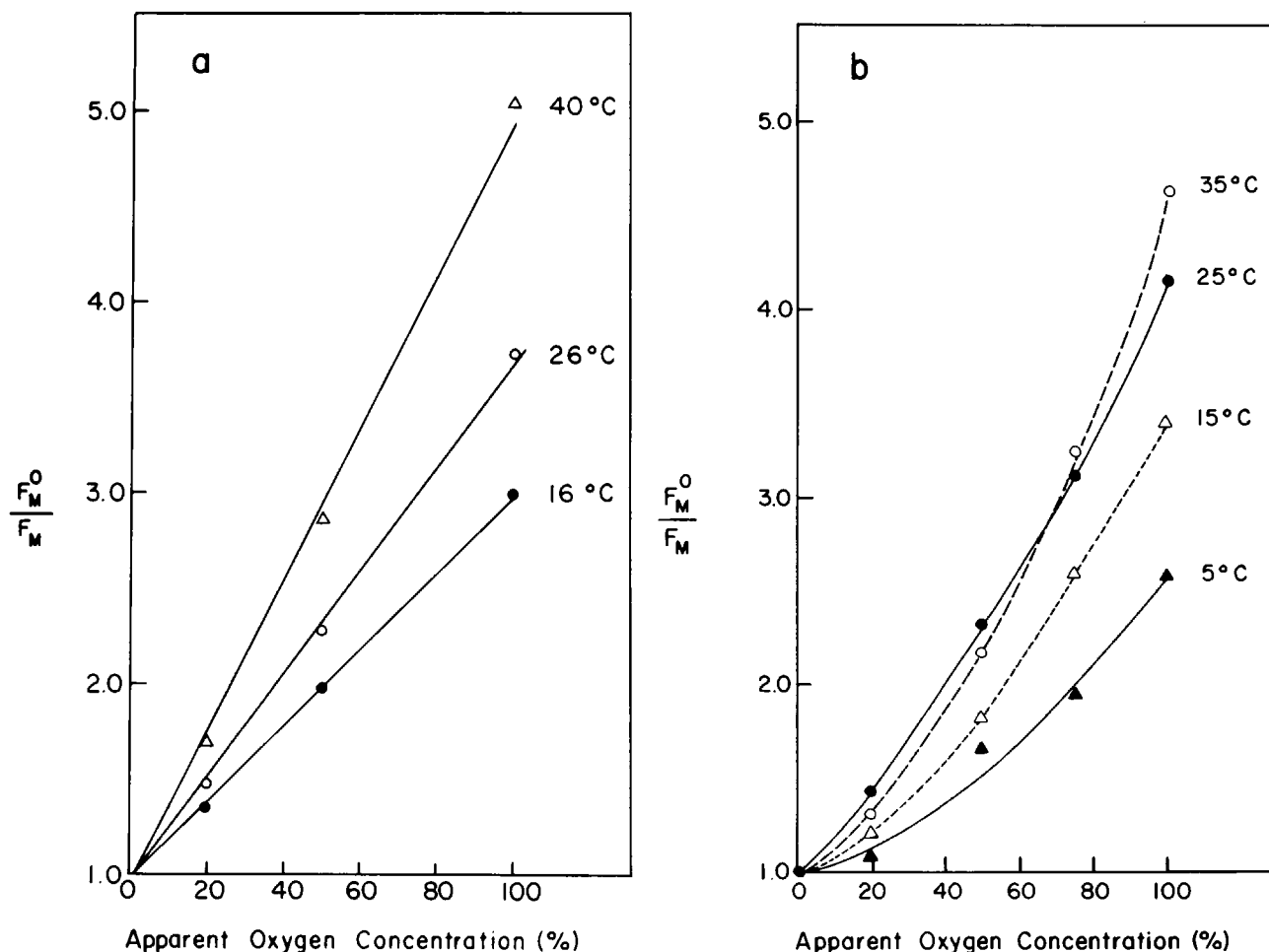


FIGURE 2 (a) Relation between F_M^0/F_M and apparent oxygen concentration for 0.84 mol % Pyr-SPM in POPC multilamellar vesicles at three different temperatures. *b* is the case for 0.9 mol % Pyr-PC in POPC multilamellar vesicles.

measured lifetime in the absence of excimer and in the absence of oxygen, $\tau_{0\%}^0(M)$. The resulting values of $\tau_{0\%}^0(M)$ for Pyr-SPM in POPC are presented in the inset of Fig. 3 *a*. A linear decrease of $\tau_{0\%}^0(M)$ with increasing temperature is evident.

Fig. 3 *b* is the data for Pyr-PC in POPC multilamellar vesicles. As before, a linear plot of $1/\tau_{0\%}^0(M)$ vs. mole percent Pyr-PC is obtained. The resulting $\tau_{0\%}^0(M)$ values

also linearly decrease with increasing temperature as shown in the inset of Fig. 3 *b*.

Temperature Dependence of k_{dm}

The data for intensity quenching (Table I) and the data in the inset of Fig. 3 *a* were used to calculate k_{dm} using Eq. 11. The calculated k_{dm} values at various temperatures for Pyr-SPM in POPC are plotted in Fig. 4 *a*. k_{dm} increases

TABLE I
SLOPES OF THE F_M^0/F_M VS $[O_2]$ PLOTS IN VARIOUS CASES

| Temperature (°C) | | | | | | | |
|----------------------------|---------|---------|---------|---------|---------|---------|---------|
| 16 | 26 | 32 | 36 | 40 | 45 | 50 | 54 |
| 4.07 mol % Pyr-SPM in POPC | | | | | | | |
| 0.0142 | 0.0192 | 0.0210 | 0.0219 | 0.0220 | 0.0223 | 0.0219 | 0.0210 |
| ±0.0028 | ±0.0020 | ±0.0011 | ±0.0015 | ±0.0020 | ±0.0026 | ±0.0034 | ±0.0040 |
| 0.84 mol % Pyr-SPM in POPC | | | | | | | |
| 0.0202 | 0.0297 | 0.0358 | 0.0390 | 0.0415 | 0.0423 | 0.0418 | 0.0420 |
| ±0.0024 | ±0.0037 | ±0.0024 | ±0.0027 | ±0.0035 | ±0.0043 | ±0.0042 | ±0.0046 |

TABLE II
EXAMPLES OF MEASURED PHASE, $\tau(\phi)$, AND
MODULATION, $\tau(M)$, LIFETIMES OF PYR-PC IN POPC
MULTILAMELLAR VESICLES IN THE ABSENCE
OF OXYGEN

| Mole percent Pyr-PC | Temperature | $\tau(\phi)$ | $\tau(M)$ |
|------------------------|-------------|----------------|---------------|
| | | <i>ns</i> | <i>ns</i> |
| 0.4 | 15°C | 138.66 ± 20.49 | 163.47 ± 3.20 |
| | 36 | 112.56 ± 20.69 | 122.96 ± 2.03 |
| | 55 | 94.90 ± 15.35 | 97.19 ± 2.97 |
| 1.9 | 15 | 100.97 ± 8.26 | 117.26 ± 2.37 |
| | 35 | 63.55 ± 1.18 | 76.42 ± 0.70 |
| | 54 | 46.95 ± 3.07 | 50.43 ± 0.79 |
| 2.3 | 15 | 81.48 ± 3.64 | 99.06 ± 1.17 |
| | 35 | 55.23 ± 2.30 | 67.20 ± 0.88 |
| | 54 | 38.47 ± 0.43 | 44.38 ± 0.23 |

monotonically with temperature from 16 to 40°C, becomes insensitive to temperature from 40 to 50°C and increases again at 54°C. In contrast, no abrupt change in k_{dm} is seen (Fig. 4 *b*) for Pyr-PC in POPC multilamellar vesicles; k_{dm} linearly changes with temperature.

DISCUSSION

Pyrene-lipid monomer, like the monomer of pyrene and other pyrene derivatives, has a very long fluorescence lifetime in the absence of oxygen. Since $\tan \phi = 2\pi f\tau$ (Spencer and Weber, 1969), the phase shift of pyrene-lipid monomer with respect to the excitation is usually quite large, even at 6 MHz modulation frequency (the lowest available modulation frequency in the SLM 4800S phase modulation fluorometer; SLM Instruments, Inc.). Under these conditions, the shorter-lifetime component in the system will contribute substantially to $\tau(\phi)$ at a modulation frequency of 6 MHz but less so at a frequency of 1 MHz. This point is clearly illustrated in Table III.

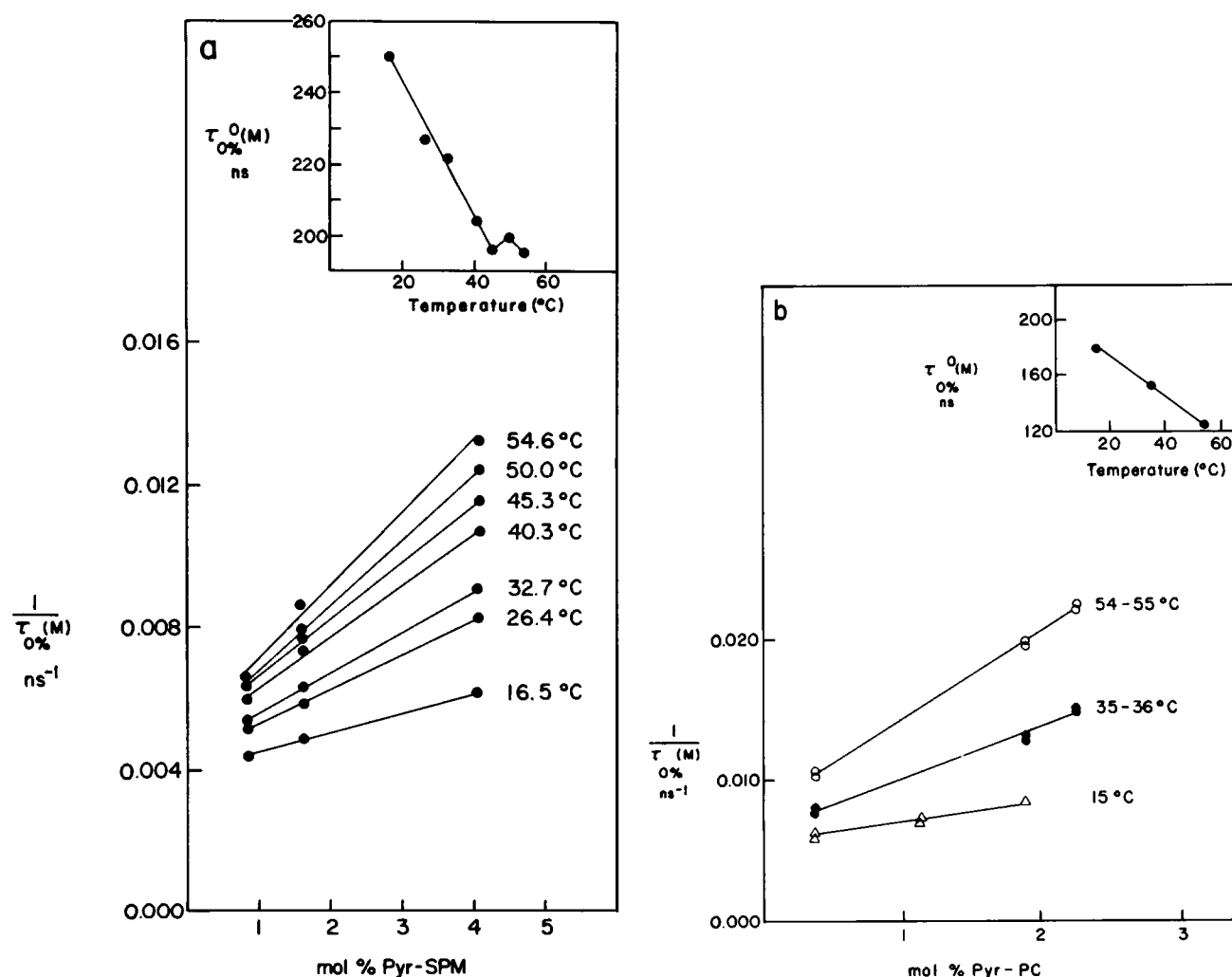


FIGURE 3 (a) Reciprocal of modulation lifetime of Pyr-SPM monomer in the absence of oxygen as a function of mole percent Pyr-SPM in POPC vesicles. Inset: modulation lifetime in the absence of oxygen and in the absence of excimer, $\tau_0(M)$ as a function of temperature. *b* is the case for Pyr-PC in POPC multilamellar vesicles.

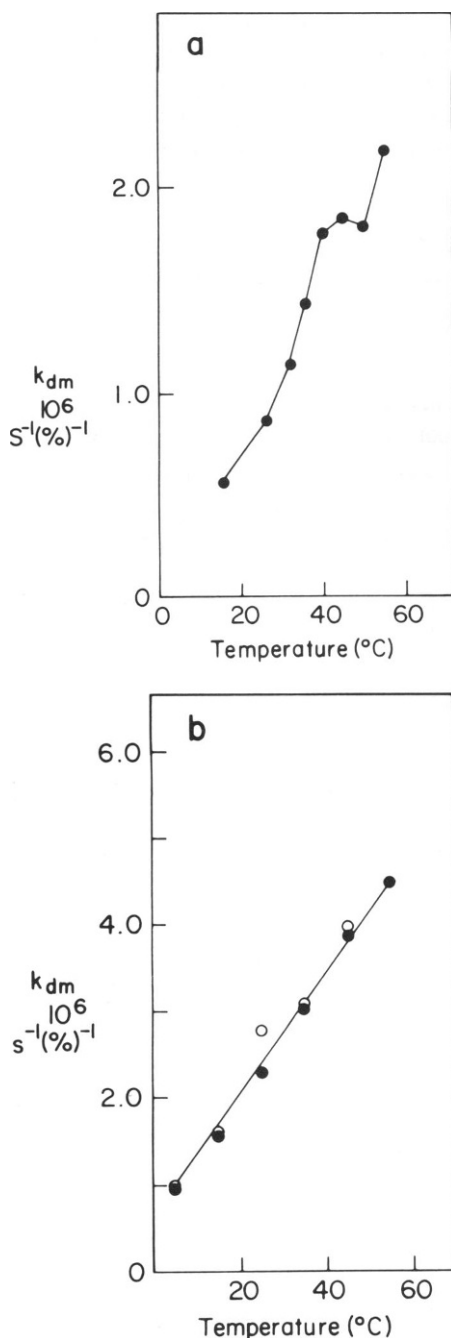


FIGURE 4 (a) Dependence of k_{dm} on temperature for the case of Pyr-SPM in POPC multilamellar vesicles. k_{dm} s were determined from the data of $M_{01} = 0.84$ mol % and $M_{02} = 4.07$ mol %. (b) Dependence of k_{dm} on temperature for the case of Pyr-PC in POPC multilamellar vesicles. k_{dm} s were determined from the data of $M_{01} = 0.23$ mol % and $M_{02} = 1.34$ mol % (dark circles) or from $M_{01} = 0.9$ mol % and $M_{02} = 4.0$ mol % (open circles).

Table III gives simulated $\tau(\phi)$ and $\tau(M)$ values, which were calculated by the method of Weber (1981). Assuming that the system consists of two components with $\tau_1 = 160$ ns (the lifetime of pyrene-lipid monomer) and $\tau_2 = 0.1$ or 0.01 ns (the lifetime of Raleigh scattered light from the lipid dispersions), the theoretical $\tau(\phi)$ and $\tau(M)$ for vari-

TABLE III
SIMULATED PHASE AND MODULATION
LIFETIMES

| Modulation frequency | Assigned | | | Calculated | |
|----------------------|----------|----------|--------|--------------|-----------|
| | τ_1 | τ_2 | f_2 | $\tau(\phi)$ | $\tau(M)$ |
| MHz | ns | ns | | ns | ns |
| 18 | 160 | 0.01 | 0.0005 | 137.42 | 159.99 |
| | | | 0.005 | 60.37 | 159.33 |
| | | | 0.015 | 26.67 | 154.35 |
| 6 | 160 | 0.01 | 0.0005 | 157.06 | 160.00 |
| | | | 0.005 | 134.70 | 159.92 |
| | | | 0.015 | 101.96 | 159.33 |
| 3 | 160 | 0.01 | 0.0005 | 159.20 | 160.00 |
| | | | 0.005 | 152.28 | 159.98 |
| | | | 0.015 | 138.68 | 159.82 |
| 1 | 160 | 0.01 | 0.0005 | 159.84 | 160.00 |
| | | | 0.005 | 158.40 | 160.00 |
| | | | 0.015 | 155.25 | 159.96 |
| 18 | 160 | 0.1 | 0.0005 | 137.44 | 159.98 |
| | | | 0.005 | 60.43 | 159.19 |
| | | | 0.015 | 26.74 | 153.96 |
| 6 | 160 | 0.1 | 0.0005 | 157.06 | 159.99 |
| | | | 0.005 | 134.72 | 159.91 |
| | | | 0.015 | 101.99 | 159.28 |
| 3 | 160 | 0.1 | 0.0005 | 159.20 | 160.00 |
| | | | 0.005 | 152.28 | 159.97 |
| | | | 0.015 | 138.69 | 159.80 |
| 1 | 160 | 0.1 | 0.0005 | 159.84 | 160.00 |
| | | | 0.005 | 158.40 | 159.99 |
| | | | 0.015 | 155.25 | 159.96 |

ous intensity fractions of the short component (f_2) at different modulation frequencies were calculated. It is clear from Table III that the difference between $\tau(\phi)$ and $\tau(M)$ decreases with the decrease of modulation frequency and with the decrease of the intensity fraction of the short component. Hence, the origin of the difference between $\tau(M)$ and $\tau(\phi)$ observed in Table II is probably due to the presence of a trace amount of a very short component, most likely the Rayleigh scattered light. Although, as shown in Table III, use of a modulation frequency of 1 MHz instead of 6 MHz can narrow the difference between $\tau(\phi)$ and $\tau(M)$, it was found unnecessary to do so here. As shown in Table III, $\tau(M)$ is not significantly affected by modulation frequency between 18 and 1 MHz, provided that the short-lifetime component (0.1–0.01 ns) contributes little, <1.5%, to the total emission. In fact, $\tau(M) \sim \tau (= 160$ ns) in all cases examined in Table III. For the purpose of k_{dm} determination at different temperatures, it is immaterial how the difference between $\tau(\phi)$ and $\tau(M)$ actually occurs. What is required in applying Eq. 11 is the temperature dependence of monomer lifetime in the absence of oxygen and in the absence of excimer. In principle, either the temperature dependence of the modulation-measured lifetime or that of the phase-measured lifetime will suffice. Since under our experimental conditions, $\tau(M)$ shows good accuracy as shown in Table III, we chose it as the lifetime in our k_{dm} calculations.

The temperature dependence of k_{dm} can reflect the molecular organization of the bilayer. Consider first the simple case (a) in which the pyrene-labeled lipid is randomly dispersed throughout the matrix lipid over the temperature range of observation. If this is the case, k_{dm} is solely controlled by the physical state of the matrix lipid, the principal component. If the matrix lipid is liquid crystalline over the temperature range of observation, then k_{dm} should show a smooth monotonic increase with increasing temperature. This situation is the case for Pyr-PC in POPC as shown in Fig. 4 b. POPC has a phase transition temperature of -5°C (Silvius, 1982). Pyr-PC has a phase transition temperature at $\sim 14.8^{\circ}\text{C}$ (Hresko, R. C., M. Wong, and T. E. Thompson, unpublished data).

Consider now, however, a more complicated case (b) in which the pyrene-labeled lipid and the matrix lipid become laterally phase separated at some temperature to give a phase rich in the pyrene-labeled lipid. In this case a sharp break in the temperature dependence of k_{dm} would occur at the temperature of lateral phase separation. The break would be caused by the concentration change accompanying lateral phase separation and/or by a change of state in the phase containing the pyrene-labeled lipid. This second situation (b) appears to be the case observed with the Pyr-SPM/POPC system. Pyr-SPM exhibits a gel-liquid crystalline phase transition at $\sim 40^{\circ}\text{C}$ (Hresko, R. C., M. Wong, and T. E. Thompson, unpublished observations). The k_{dm} data in Fig. 4 a show a break at about that temperature suggesting that Pyr-SPM rich, gel-like domains or clusters exhibit below this temperature in a liquid-crystalline POPC matrix. This interpretation is consistent with the suggestion made by Frank et al. (1983) based on the temperature dependence of interbilayer transfer rates of Pyr-SPM in an identical vesicle system.

The contrasting behavior of Pyr-PC and Pyr-SPM in POPC bilayers is also reflected in the intensity of quenching. As shown in Fig. 2, upward curvature is apparent in the case of Pyr-PC in POPC when the pyrene concentration and the temperature are high. But in the case of Pyr-SPM, no curvature is seen in the range of pyrene concentrations or temperature examined.

Positive curvature in Stern-Volmer plots can be explained in several ways. Traditionally, an upward curve is attributed to a strong contribution from static quenching (Bowen and Metcalf, 1951; Vaughan and Weber, 1970). However, an upward curve can also result if the quenching rate constant, $k_{t,m}^*$, is quencher-concentration dependent (Keizer, 1983). Thus the difference in the oxygen quenching between Pyr-PC/POPC system and the Pyr-SPM/POPC system may be the result of a relatively higher oxygen concentration in the vicinity of Pyr-PC than in the vicinity of Pyr-SPM. This conclusion is strengthened by the following consideration. Pyr-PC/POPC is clearly a randomly mixed liquid crystalline system whereas Pyr-SPM/POPC, a gel-like phase rich in Pyr-SPM, exists below 40°C . Recent experiments by Subczynsky and Hyde

(1983), who used spin-label methods to determine the oxygen partition coefficient in dimyristoyl phosphatidylcholine dispersion, clearly show that O_2 solubility in the gel phase is much less than in the liquid crystalline state.

Very recently Gratton et al. (1984) proposed a model in which the distribution of quenchers among target macromolecules also may affect the shape of Stern-Volmer plots. However, Jameson et al. (1984) pointed out that when the quencher concentration is very low, the quencher distribution among target molecules is unimportant. The quencher concentrations used in our study were even lower than the low quencher concentrations used by Jameson et al. (1984). Thus, the distribution of oxygen among vesicles is not of importance in our studies.

APPENDIX

The relatively long lifetime of pyrene fluorescence renders it particularly susceptible to quenching by oxygen (Vaughan and Weber, 1970; Geiger and Turro, 1975; Fischkoff and Vanderkooi, 1975). The excitation and emission processes of pyrene or its derivatives in the presence of oxygen are schematically described in Fig. 5. The kinetic equations of monomer and excimer emission are as follows:

$$\frac{dM^*}{dt} = -(\Gamma_m + k_{dm}M_o + k_{t,m}^*[O_2])M^* + f(t) \quad (\text{A1})$$

$$\frac{dM_2^*}{dt} = -(\Gamma_e + k_{t,e}^*[O_2])M_2^* + k_{dm}M_oM^*, \quad (\text{A2})$$

where M_2^* = excimer, Γ_e = rate of emission of excimer ($= 1/\tau_e$), M_o = total monomers, M^* = excited monomer, Γ_m = rate of emission of monomer ($= 1/\tau_m$), $k_{t,m}^*$, or $k_{t,e}^*$ = rate of quenching by oxygen for monomer and excimer, respectively, k_{dm} = rate of association of M^* with M or the rate of excimer formation, and $f(t)$ = the excitation function. Note that τ_m and τ_e include both radiative (k_{fm} and k_{fe} , Birks et al., 1963) and nonradiative rate constants.

Under stationary excitation conditions, since

$$\frac{dM^*}{dt} = 0, \quad \frac{dM_2^*}{dt} = 0, \quad \text{and } f(t) = \text{constant}, \quad (\text{A3})$$

from Eqs. A1 and A2 it follows that

$$M^* = M_2^* \frac{(\Gamma_e + k_{t,e}^*[O_2])}{k_{dm}M_o} \quad (\text{A4})$$

and

$$M^* = \frac{f(t)}{\Gamma_m + k_{dm}M_o + k_{t,m}^*[O_2]} \quad (\text{A5})$$

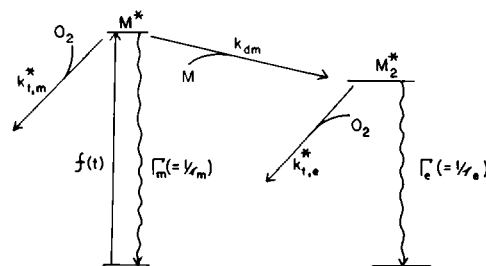


FIGURE 5 Schematic diagram of photophysics of pyrene or pyrene derivatives in the presence of oxygen.

As a result, the monomer emission, F_m , can be written as

$$F_m = \Gamma_m \cdot M^* = \frac{f(t)}{1 + k_{dm}M_o\tau_m + k_{t,m}^*\tau_m[O_2]} \quad (A6)$$

Let F_m^o be the monomer emission in absence of oxygen. Then

$$\frac{F_m^o}{F_m} = 1 + \frac{k_{t,m}^*\tau_m}{1 + k_{dm}M_o\tau_m} [O_2]. \quad (A7)$$

According to this scheme, then, a plot of F_m^o/F_m should be a straight line with a slope dependent upon k_{dm} , M_o , τ_m , and $k_{t,m}^*$. Assuming that k_{dm} and the oxygen quenching rate constant at a given temperature are independent of the concentration of pyrene moiety, the ratio of the slopes at two different monomer concentrations, M_{o1} and M_{o2} , is given by

$$R = \frac{\text{slope 1}}{\text{slope 2}} = \frac{1 + k_{dm}M_{o2}\tau_m}{1 + k_{dm}M_{o1}\tau_m}, \quad (A8)$$

which can be rearranged to

$$k_{dm} = \frac{1 - R}{\tau_m(RM_{o1} - M_{o2})}. \quad (A9)$$

Thus, the ratio of slopes, combined with the experimentally determined monomer lifetime, should yield the value of k_{dm} . Here, it must be noted that τ_m is the monomer lifetime in the absence of oxygen and at the same time in the absence of excimer. This monomer lifetime is now designated as τ_m^o , where the subscript 0 and the superscript 0 have been introduced to indicate that the lifetime is the lifetime in the absence of oxygen and in the absence of excimer.

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