Dipyrenylphosphatidylcholines as membrane fluidity probes

Relationship between intramolecular and intermolecular excimer formation rates

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ABSTRACT In the intramolecular excimeric membrane probe, dipyrenylphosphatidylcholine (dipy$_n$ PC), pyrene moieties are linked to the terminal carbons of the two acyl chains, each of which contains n carbons. We show here how the probe intramolecular excimer production rate, K, may be determined from the excimer/monomer intensity ratio, r_I, by making use of the fluorescence titrations of the related monopyrenyl probe, py$_n$ PC, analyzed according to the milling crowd model. r_I and the rate K of dipy$_{10}$ PC in four model membrane systems were measured over a wide temperature range and both parameters are shown to be sensitive functions of the lateral fluidity of the host matrix. A model for relating the intramolecular and intermolecular excimer formation rates is proposed according to which both processes are limited by the reorientational rate of the pyrene moiety. Above the fluid-gel transition temperature, T_c, the diffusion rate (f) of the monopyrenyl probe (py$_n$ PC) is accordingly related to K by: $p_E \approx K/(K + \frac{1}{2}f + \tau_M^{-1})$, where p_E is the probability of excimer formation between nearest neighbor py$_n$ PC probes, and τ_M is the monomer lifetime. Values of p_E derived in this way are found to be consistent with p_E values derived from the milling crowd analysis of fluorescence yield titration experiments. K for dipy$_{10}$ PC in DMPC multibilayers ranges from 0.21×10^7 s^{-1} at 10°C in the gel phase, to 5.7×10^7 s^{-1} at 60°C in the fluid phase, whereas the lateral diffusion coefficient, D, for py$_{10}$ PC in the same bilayers ranged from 8 to 34 μm^2 s^{-1}, when calculated with $D = fL^2/4$, L being the average lipid–lipid spacing of the host membrane. Above T_c and at the same reduced temperature, $(T - T_c)/T_c$, both f for py$_{10}$ PC, and K for dipy$_{10}$ PC were found to have relative magnitudes in the order: DPPC > DMPC > POPC > DOPC. This and the similarity of the activation energies for f and K suggest that the rotation of the the pyrene moiety is the rate-limiting step for both the lateral mobility of py$_{10}$ PC and intramolecular excimer formation in dipy$_{10}$ PC.

INTRODUCTION

Fluorescent lipid analogues have long been used to explore the dynamic and structural properties of membranes. Among them are photobleaching probes employed in photobleaching recovery (FPR) measurements of long-range (≈1 μm) lipid diffusivity (Vaz et al., 1982), rotational probes, whose emission polarization is a measure of the probe orientational mobility (Shinitzky and Henkart, 1978), and intermolecular excimeric probes, whose rate of excimer production provides a measure of their translational mobility (Galla et al., 1979; Eisinger et al., 1986). While these and similar probes all provide measures of "membrane fluidity," the dynamical parameters reported by each probe differ and are not necessarily related to each other (Kleinfeld et al., 1981). On the other hand, it is reasonable on theoretical grounds, that the concept of membrane packing and the "free volume" of a membrane (i.e., the fractional volume not occupied by its constituent molecules), is intimately connected with its lateral fluidity, i.e., the diffusivity of lipid analogue probes in the membrane. The vacancy model for diffusive mobility is in fact based on the concept that the mobility of a molecule is directly related to the frequency with which a vacancy of sufficient size is created in its neighborhood (Cohen and Turnbull, 1959). It has indeed been demonstrated that when the free volume of a membrane is reduced by the application of high pressure, lateral membrane fluidity is dramatically reduced (Eisinger and Scarlata, 1987).

Dipyrenyl phosphatidylcholine (dipy$_n$ PC) is a lipid analogue membrane probe in which the terminal carbons of both acyl chains, n carbons in length, are conjugated to pyrene moieties. The intramolecular excimer formation rate of dipy$_n$ PC molecules is a measure of the free volume available in the host matrix, because two nearest neighbor pyrene molecules, one in the excited state, can form an excimer only if they have appropriate relative orientations.

It is shown below how K, the rate of excimer formation of dipy$_n$ PC probes, can be derived from the measured excimer/monomer fluorescence ratio, by making use of experimental parameters of monopyrenyl PC probes (py$_n$ PC), with the same length pyrenyl acyl chain. The analysis is based on the so-called milling crowd model which is described in a companion paper (Sassaroli et al.,

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1990), and yields f, the probe diffusion rate. The two rates, K and f, were measured in several model membrane systems and were found to be linearly related and to have approximately the same activation energies, suggesting that the intermolecular and intramolecular excimer formation processes have a common rate-limiting mechanism.

EXPERIMENTAL METHODS

Dimyristoylphosphatidylcholine (14:0, 14:0 acyl chains; DMPC), dipalmitoyl-PC (16:0, 16:0; DPPC) 1-palmitoyl-2-oleoyl-PC (16:0, 18:1; POPC) and dioleoyl-PC (18:1, 18:1; DOPC) were obtained from Avanti Polar Lipids, Birmingham, AL. The stock solutions of these lipids at a concentration of ~10 mM were kept in a freezer in chloroform/methanol solvent (CM, 4:1 by volume). Their concentrations were determined by use of a phosphate assay (Bartlett, 1959). 1-Palmitoyl-2-(1'-pyrenedecanoyl)-PC (py_{10} PC) was obtained from Molecular Probes, Inc., Eugene, OR. Di-(1'-pyrenedecanoyl)-PC ($dipy_n$ PC) was synthesized by methods described previously (Patel et al., 1979). The monopyrenyl and dipyrenyl probes were dissolved in the CM solvent at a concentration of ~100 μM and concentrations were determined spectrofluorometrically, using 42,000 cm^{-1} as the molar extinction coefficient of the pyrene moieties at 342 nm.

Medium size multilamellar vesicles (MLV) were prepared by drying a mixture of phospholipids (100 nmol) and the pyrenyl phospholipid probes (0.1–10 mol % of total lipid), each solubilized in chloroform, under a stream of nitrogen gas and thereafter for 16 h under reduced pressure to remove all solvent. The lipids deposited on the wall of the test tube were then hydrated by the addition of 2 ml of buffer (10 mM Hepes, pH 7.2, 5 mM KCl, 140 mM NaCl) and vortexed intermittently. The suspensions were then placed in an ultrasonic bath (Branson Inc., Danbury, CT; 100 W power) for 15 min. The temperature during hydration was maintained well above the fluid-gel transition temperature (35°C for DMPC, POPC, and DOPC, 55°C for DPPC).

Steady-state fluorescence measurements were made with samples equilibrated in air, using 1-cm cuvettes in a steady-state SLM-8000 spectrofluorometer (SLM Instruments Inc., Urbana, IL), equipped with a thermostatically controlled cuvette holder. Excitation and emission slits were 4 nm in width. The excitation wavelength was 320 nm and the monomeric and excimeric emissions were measured at 378 and 480 nm, respectively. Fluorescence lifetimes were measured using samples with probe ratios of 0.001, by use of a photon counting time-resolved fluorometer with short (picosecond) excitation pulses supplied by a mode-locked Nd-YAG-pumped dye laser. The errors in the quoted average lifetimes are ~5%.

EXCIMER FORMATION RATE OF INTRAMOLECULAR EXCIMERIC PROBES

Whereas the intermolecular excimer formation rate is a function of the concentration and mobility of the excimeric molecules, the rate of intramolecular excimer formation, K, is, at sufficiently low probe levels, independent of probe concentration. K of $dipy_n$ PC does however depend on the lateral and rotational mobility of its pyrene moieties and on the constraints imposed by the molecular environment of the probe. The ratio of excimeric to monomeric emission intentisies $r_I = I_E/I_M$, can be measured easily and with considerable accuracy and reflects the probability that the two pyrene moieties attain the separation and relative orientation required for excimer formation, within the lifetime of the excited pyrene monomer ($\tau_M \approx 10^{-7}$ s).

Whereas r_I of $dipy_n$ PC provides a useful empirical measure for comparing the membrane fluidity of different host systems, the absolute rate of excimer formation, K, is a more fundamental parameter of the dynamics of the probe which can ultimately be compared to molecular dynamical models. This section provides a protocol for deriving K from r_I and other experimental parameters.

The kinetic equations for an intramolecular excimeric probe are given below. They are formally analogous to those of intermolecular ones (Eisinger et al., 1986; Sassaroli et al., 1990), except that K is concentration-independent.

$$A + A^* \xrightarrow{K} (AA)^* \tag{1}$$

$$A^* \xrightarrow{k_M,\, k'_M} A + h\nu_M \tag{2}$$

$$(AA)^* \xrightarrow{k_E,\, k'_E} 2A + h\nu_E. \tag{3}$$

Here A and A* represent the ground and excited-state pyrene moieties, k and k' are the radiative and nonradiative deexcitation rates with the subscripts M and E denoting photon ($h\nu$) emission from excited monomers, A*, and excimers (AA)*, respectively. The monomeric and excimeric quantum yields are, accordingly,

$$\Phi_M = \frac{k_M}{k_M + k'_M + K} \tag{4}$$

$$\Phi_E = \frac{k_E}{k_E + k'_E}\,\frac{K}{k_M + k'_M + K}, \tag{5}$$

where the excimer dissociation rate, K_d, is assumed to be negligible compared with the excimer decay rate ($K_d \ll k_E + k'_E$). If this is not the case, the corrected excimer formation rate is

$$K_{corr} = K\,\frac{k_E + k'_E + K_d}{k_E + k'_E}. \tag{6}$$

For an isolated PC probe with only one of its acyl chains linked to a pyrene moiety, in the same environment as the dipyrenyl probe $K = 0$ and its monomeric quantum yield is (c.f. Eq. 4)

$$\Phi^*_M = \frac{k_M}{k_M + k'_M}. \tag{7}$$

Similarly, the quantum yield of a probe in which the pyrenyl moieties are so positioned that every excitation leads to the formation of an excimer (i.e., $K \gg k_M + k'_M$) is, according to Eq. 5,

$$\Phi_E^* = \frac{k_E}{k_E + k'_E}. \tag{8}$$

Φ_M^* and Φ_E^* are the limiting quantum yields of monopyrenyl probes at very low ($x \ll 1$) and very high ($x \approx 1$) probe concentration, conveniently expressed as the probe/lipid molar ratio, x.

With the quantum yield ratios,

$$r_\Phi = \Phi_E/\Phi_M \quad \text{and} \quad r_\Phi^* = \Phi_E^*/\Phi_M^*, \tag{9}$$

it follows from Eqs. 4–7 that

$$K = \frac{r_\Phi}{r_\Phi^*}\tau_M^{-1}, \tag{10}$$

where $\tau_M = (k_M + k'_M)^{-1}$ is the lifetime of the excited isolated monomer ($\approx 10^{-7}$ s). It is shown in a companion paper (Sassaroli et al., 1990), that

$$r_\Phi^* = r_\Phi(x_0), \tag{11}$$

where x_0 is the probe ratio for which the excimer formation rate is equal to the monomeric fluorescence decay rate (c.f. Eq. 14; for py_{10} PC in DMPC, $x_0 \approx 0.05$).

It is, however, not necessary to measure absolute quantum yields to determine K. As long as the fluorescence spectra of the monopyrenyl and dipyrenyl probe systems are measured in the same fluorometer, the excimer and monomer intensities, I_E and I_M, measured at particular wavelengths (typically 480 and 378 nm) are proportional to the corresponding quantum yields. By writing the excimeric and monomeric intensities of the monopyrenyl probes at the critical probe ratio as $I_E(x_0)$ and $I_M(x_0)$,

$$r_I^* = r_I(x_0) \equiv \frac{I_E(x_0)}{I_M(x_0)}, \tag{12}$$

so that from Eqs. 10 and 12,

$$K = \frac{r_I}{r_I(x_0)}\tau_M^{-1}. \tag{13}$$

The critical probe ratio x_0 is readily obtained from the concentration dependence of the monomeric fluorescence yields, (c.f. Fig. 3), because by definition,

$$J_M(x_0) = J_M^*/2, \tag{14}$$

where relative yields are here conveniently expressed as $J_M(x) = I_M(x)/x$ and $J_E(x) = I_E(x)/x$, with J_M^* and J_E^* being the limiting yields for $x \ll 1$ and $x \approx 1$, respectively. With x_0 known, the intramolecular excimer formation rate may be calculated from the intensity ratios for the intermolecular and intramolecular probes, by use of Eq. 13.

Alternatively, the monomer fluorescence yield titration data may be fitted to the equation

$$J_M(x) = J_M^* \frac{1}{1 + f\tau_M/n(p_E, x)}, \tag{15}$$

which is based on the milling crowd model for lateral diffusion (c.f. Eq. 23a of reference [Sassaroli et al., 1990]). From the best fit one obtains optimal values of J_M^*, the diffusion rate f, and of p_E, the probability that an excited probe which is a nearest neighbor to a probe in its ground state, forms an excimer during a time f^{-1}. An equivalent fitting procedure may be applied to the excimer fluorescence yields, or to r_I, as described in a companion paper which also gives the functional values of $n(p_E, x)$, the average number of steps taken by a probe before it forms an excimer, as derived from computer simulations of random walks (Sassaroli et al., 1990).

In the present investigation, the parameters K and r_I were determined for the dipyrenyl probe $dipy_{10}$ PC in several bilayer systems, as was $r_I(x_0)$ for its analogue monopyrenyl probe, py_{10} PC.

RELATIONSHIP BETWEEN K AND PROBE DIFFUSIVITY

It is useful to consider the kinetics of excimer formation among diffusing membrane probes as occurring in two steps: first, the diffusing probes form a nearest neighbor pair, $(A,A^*)_n$, which serves as an intermediate reaction complex from which an excimer may be formed:

$$\begin{array}{ccccc} A + A^* & \underset{f_r}{\overset{f_n}{\rightleftharpoons}} & (A, A^*)_n & \underset{K_{nr}}{\overset{K_n}{\rightleftharpoons}} & (AA)^* \\ \downarrow & & \downarrow & & \downarrow \\ A + h\nu_M & & A + h\nu_M & & 2A + h\nu_E \end{array} \tag{16}$$

In this reaction scheme, f_n, the rate at which probes become nearest neighbors, is a function of probe concentration and is, according to the milling crowd, model, equal to $f/n(1, x)$ (Sassaroli et al., 1990). The reverse rate, f_r, is the rate at which a nearest neighbor pair diffuses apart and K_n is the rate at which excimers are formed from a $(A, A^*)_n$ pair. In the steady-state analysis presented here, K_{nr} will be considered negligible compared with the excimer decay rate but its effect will be included in a later study of the time dependence of the monomeric and excimeric fluorescence.

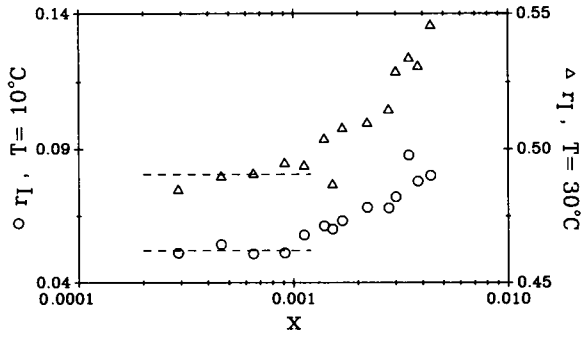

FIGURE 1 The concentration dependence of the excimer/monomer fluorescence ratio (r_I) of dipy$_{10}$ PC in DMPC multilamellar vesicles, in the gel and fluid phase, at 10 and 30°C, respectively. r_I is seen to be concentration independent for $x \leq 0.001$.

Note that in the milling crowd model two nearest neighbor probes separate at a rate of approximately one half the diffusion rate, i.e., $f_r \approx f/2$. This follows from the fact that each probe can take steps in six distinct directions, but that in 17 of the 36 possible combinations the probes remain nearest neighbors, i.e., the random steps taken by each probe are correlated with a correlation factor of very nearly one-half. It follows from the kinetic scheme of Eq. 16 that the probability that the $(A, A^*)_n$ pair forms an excimer is

$$p_E \approx \frac{K}{K + \frac{1}{2}f + \tau_M^{-1}}, \qquad (17)$$

where we have set $K_n = K$, which is equivalent to our assumption that the excimer formation rate of the nearest neighbor complex of monopyrenyl probes, $(A, A^*)_n$, is approximately the same as that of the related dipyrenyl probe. This assumption is justified if the reorientation rate of the pyrene moieties is the rate-limiting step for $(A, A^*)_n$ to become an excimer. Eq. 17 may be rewritten as

$$K \approx \tfrac{1}{2}(p_E^{-1} - 1)^{-1} f + (p_E^{-1} - 1)^{-1} \tau_M^{-1}. \qquad (18)$$

Our model for intramolecular excimer formation therefore predicts a linear relationship between K and f.

EXPERIMENTAL RESULTS

To measure the intramolecular excimer formation rate, the probes must be randomly distributed in the membrane and their concentration must be sufficiently low for intermolecular excimer formation to be negligibly low. Fig. 1 shows the concentration dependence of the excimer/monomer intensity ratio r_I of dipy$_{10}$ PC in DMPC multilamellar vesicles in the gel and fluid phase. It is concluded from the data shown there that in DMPC the contribution of intermolecular excimers is negligible for probe ratios <0.001.

Fig. 2 shows the temperature dependence of r_I of dipy$_{10}$ PC measured in four model membrane systems. These data demonstrate that r_I is a sensitive indicator of its dynamic environment, responding to the fluid-to-gel

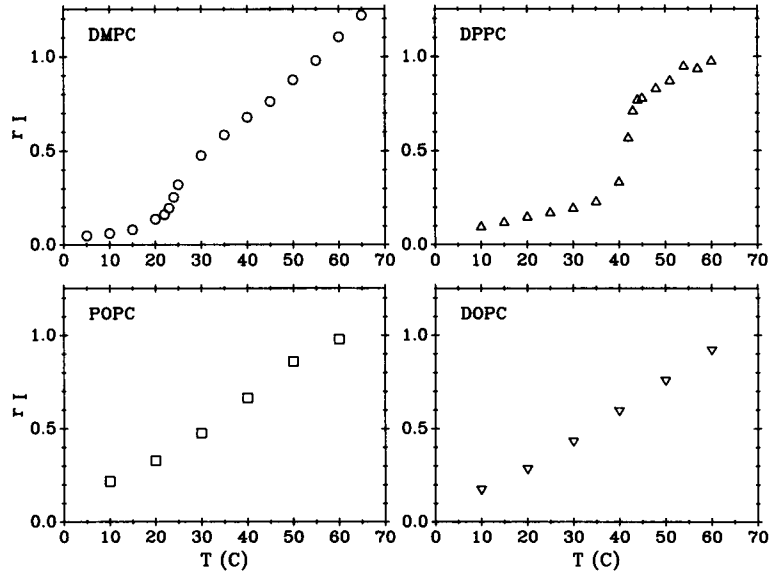

FIGURE 2. The temperature dependence of r_I for dipy$_{10}$ PC in MLV of DMPC, DPPC, POPC, and DOPC, the symbol for each being used to identify each model system in the remaining figures. Note the considerable dynamic range of r_I (a factor of 30 for DMPC between 5 and 65°C), which is due to both a longer monomer lifetime and greater membrane fluidity at the higher temperatures.

phase transition, T_c = 24 and 41°C for DMPC and DPPC, respectively. The excimer/monomer ratio has a considerable dynamic range, increasing by a factor of 30 in DMPC between 5 and 65°C; this is due to the combined effects of increased membrane fluidity and lifetime shortening, which is in turn partly due to the temperature-dependent increase in oxygen quenching of the excited monomer (Fischkoff and Vanderkooi, 1975).

To translate the intensity ratios of Fig. 2 into the absolute intramolecular excimer formation rates of $dipy_{10}$ PC in the four model membranes, it is necessary to measure in the same host membranes the diffusivity of the related monopyrenyl probe, py_{10} PC. The concentration dependences of the fluorescence yield of the intermolecular probe in DMPC, DPPC, POPC, and DOPC are shown in Fig. 3 and 4. These fluorometric titrations were measured over the same temperature range as was used for the measurement of r_1.

Values for the model-independent fluidity parameter x_0 are readily obtained from the titration data by use of Eq. 14 and are given in Table 2. The titration data were also analyzed according to the milling crowd model, as described in detail in a companion paper, to determine optimum values for $f\tau_M$ and p_E by means of Eq. 15 (Sassaroli et al., 1990). The values of τ_M, the average monomer lifetime, which were used to obtain the values of f and p_E listed in Table 1, are shown as a function of temperature in Fig. 5. The lateral diffusion coefficients, D, calculated from f and the lipid–lipid spacing of the host membranes are also listed in Table 1.

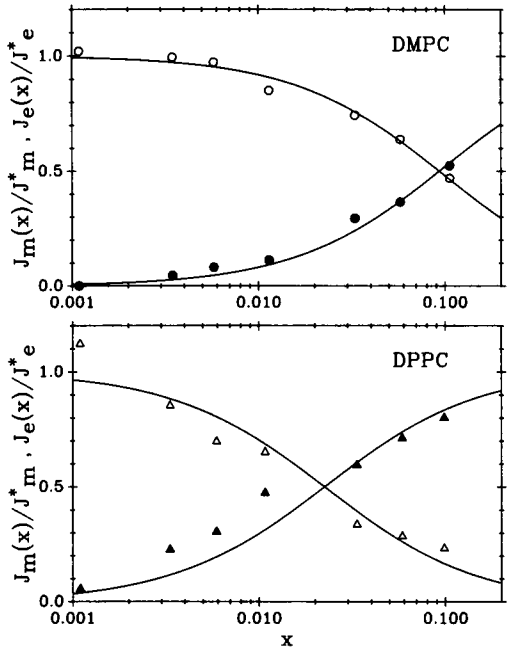

FIGURE 4 Same as Fig. 3, but for titrations for the same bilayers in the gel phase, at temperatures 15° below T_c. The curves are again the best fits obtainable by use of Eq. 15, but the quality of the fit is clearly worse than that obtained for the same bilayers in the fluid phase, particularly for DPPC. The fact that Eq. 15 cannot be used to fit the observed concentration dependence suggests that probe segregation occurs in the gel phase (c.f. Discussion).

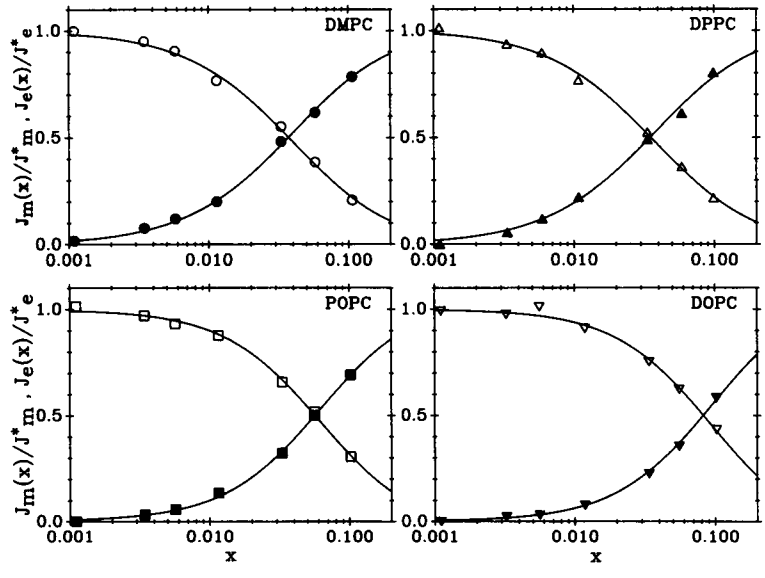

FIGURE 3 The concentration dependence of the monomer and excimer fluorescence yields of py_{10} PC in four MLV systems, measured at temperatures about 15° above the T_c of the respective bilayers. Curves represent the theoretical dependence of x according to the milling crowd model (c.f. Eq. 15), the parameters $f\tau_M$ and p_E having been chosen to provide the optimum fit to the experimental points.

TABLE 1 Dynamic parameters of py_{10}- PC and $dipy_{10}$ PC probes in four host membrane MLV, with transition temperatures and corresponding references

Host	T	x_0	f	p_E	D	K	p_E'
	°C	%	$10^7\ s^{-1}$		$\mu m^2\ s^{-1}$	$10^7\ s^{-1}$	
DMPC	10	7.8	6.0	0.25	8	0.21	0.15
24°C*	20	5.0	11.1	0.25	13	0.44	0.15
	30	5.4	14.3	0.25	22	1.4	0.26
	40	4.3	21.8	0.25	34	2.4	0.26
DPPC	42	3.7	21.6	0.25	35	2.2	0.30
41°C*	51	3.7	25.3	0.25	43	4.4	0.30
	60	3.5	32.4	0.25	56	5.7	0.30
POPC	10	6.3	4.3	0.50	8	1.0	0.34
4°C‡	20	5.6	5.8	0.50	11	1.5	0.34
	30	4.8	7.8	0.50	15	2.2	0.34
	40	4.5	10.6	0.50	20	3.0	0.34
	50	4.0	14.5	0.50	27	4.2	0.34
DOPC	10	8.2	3.1	0.50	6	0.7	0.33
−20°C§	20	7.0	4.5	0.50	9	1.1	0.33
	30	6.0	6.3	0.50	13	1.8	0.33
	40	5.1	8.9	0.50	18	2.5	0.33
	50	4.7	12.0	0.50	24	3.5	0.33

Note that only for DMPC does the temperature range cover the gel, as well as the fluid phases. Third column gives critical probe ratio (x_0) for py_{10} PC in different host systems. (It can be employed as an empirical fluidity parameter.) The diffusion frequencies, f, determined from fluorescence titrations of py_{10} PC by use of Eq. 15 is shown in column 4, with column 5 giving the value of p_E which provided the optimum fit of Eq. 15 to the data. The lateral diffusion coefficient of the monopyrenyl PC in each membrane, D, is shown in column 6. It was calculated from $D = fL^2/4$ (Berg, 1983), with L^2, the average area per phospholipid in fluid vesicles, equal to 0.62 nm^2 for DMPC at 35°C, and equal to 0.70 nm^2 for DPPC, POPC, and DOPC (Janiak et al., 1979; Demel et al., 1967; Lewis and Engelman, 1983). The weak temperature dependence of L^2 was taken into account. Molecular area used for DMPC in the gel phase is 0.48 nm^2 (Janiak et al., 1979). K for $dipy_{10}$ PC was obtained from Eq. 13, using the x_0 values of column 3 and the τ_M values shown in Fig. 4. The p_E' values in the last column are the estimates of p_E according to Eq. 17. The values for x_0, f, D, and K shown here are average values for several independent experiments, and their probable errors are estimated to be 20 percent. *Lentz et al., 1978; ‡Barenholz et al., 1976; §Op Den Kamp et al., 1975.

With r_1 of $dipy_{10}$ PC and f and $r(x_0)$ for py_{10} PC known, K was calculated by means of Eq. 13. The temperature dependence of the inter- and intramolecular rates, f and K, are shown as Arrhenius plots for the fluid phase of the four vesicle systems in Figs. 6 and 7, respectively. The slopes of the straight lines were used to determine the activation energies for the two rates, which are shown in Table 2.

The rates, K and f, of the dipyrenyl and monopyrenyl probes, respectively, are compared in Figs. 8 and 9, which show them to be linearly related to each other. From Eq. 18 and the slopes of the lines, $m = dK/df$, the probability of excimer formation for a nearest neighbor pair,

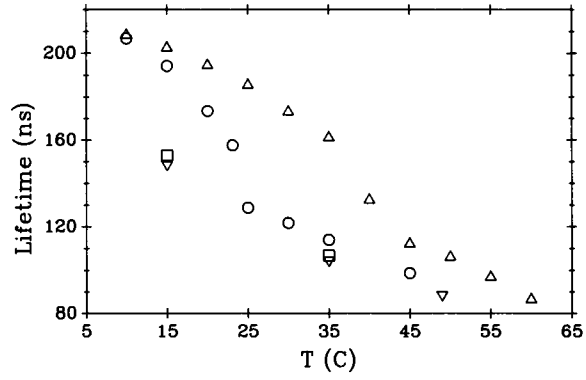

FIGURE 5 τ_M, the average excited state lifetimes of the py_{10} PC monomer, measured in different MLV systems with a probe ratio of 0.001. This is sufficiently low to preclude intermolecular excimers (c.f. Fig. 1). The symbols used for the DMPC, DPPC, POPC, and DOPC vesicles are the same as those shown in Fig. 3.

$(A, A^*)_n$, is

$$p_E \approx [(2m)^{-1} + 1]^{-1}. \quad (19)$$

Values of p_E derived from Eq. 19 for different host membranes are listed as p_E' in Table 1. These estimates are seen to be within a factor of 2 of the p_E values which provide the best fit of Eq. 15 to the yield titration data. In view of the simplicity of the model which underlines the present analysis, that is considered to be a satisfactory agreement.

DISCUSSION

The fluorescence yield titration data for py_{10} PC in fluid DPPC, POPC, and DOPC multilamellar vesicles, were well fitted by theoretical curves based on the milling crowd model for probe mobility, as was previously demonstrated for DMPC (Sassaroli et al., 1990). Below T_c, the titration data for DPPC could not be well fitted by Eq. 15, presumably because the basic assumption of our model, that the probes are randomly distributed in the host system, is not fulfilled. It is noteworthy that a reasonable agreement with the model was possible for DMPC in the gel phase (c.f. Fig. 4). This is probably due to the fact that the transition temperature of DMPC (24°C) is close to that of py_{10} PC (15°C, [Somerharju et al., 1985]), whereas the value of T_c for DPPC is well removed from that of the probe. This is thought to lead to probe segregation which violates the assumptions of the model.

The linear relationship between K and f and the similarity of the activation energy for these two rates, one intramolecular the other intermolecular, suggest that the

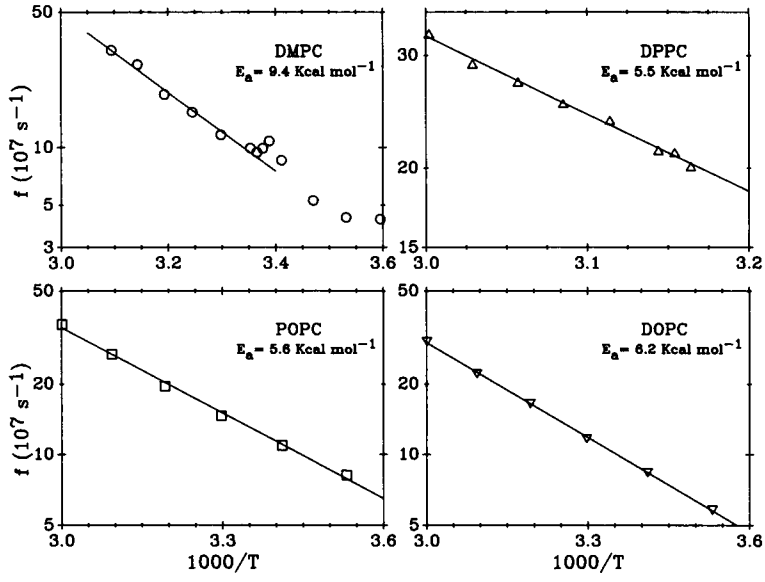

FIGURE 6 The diffusion rate, f, of py_{10} PC in four vesicle systems as a function of the inverse absolute temperature. The values of f were obtained by use of Eq. 15 with $p_E = 0.25$ and the τ_M values of Fig. 5. Note that the Arrhenius plots are straight for temperatures above T_c, the gel-fluid transition temperature. The activation energies derived from the slopes of the straight line portions are given in Table 2.

rate-limiting step for both is the reorientation of the pyrene moieties within the membrane: this motion is, in turn, controlled by the packing of the phospholipid acyl chains and the availability of sufficient free volume around the probes as described by the vacancy model for lateral diffusion (Vaz et al., 1985).

A valid comparison between the magnitudes of f and K in different host membrane systems can only be made among values measured at the same reduced temperature, $(T - T_c)/T_c$ (all temperatures being in degrees Kelvin). In Fig. 10, the rates f for py_{10} PC and K for $dipy_{10}$ PC are plotted against the reduced temperature and the

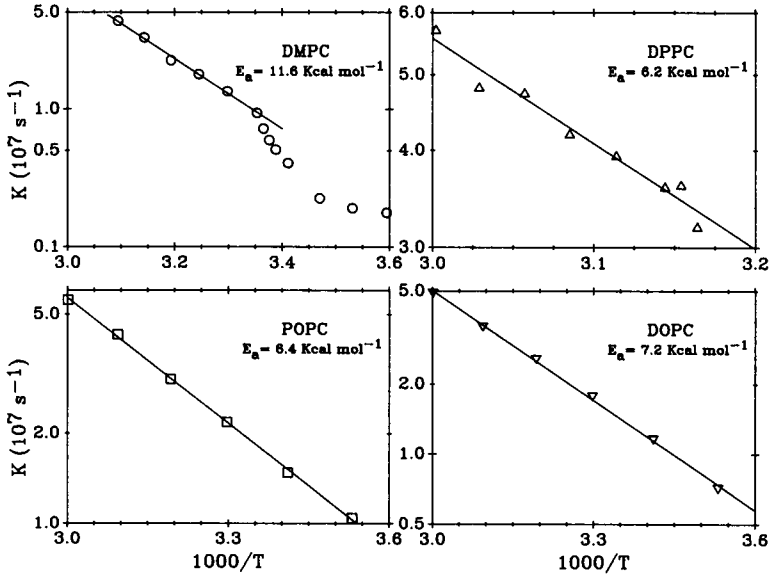

FIGURE 7 The intramolecular excimer formation rate, K, for $dipy_{10}$ PC in four vesicle systems as a function of the inverse absolute temperature. The activation energies derived from the slopes of the Arrhenius plots are given in Table 2.

TABLE 2 E_a, activation energies for diffusion rate of py_{10} PC, f, and for intramolecular excimer formation rate of $dipy_{10}$, K, in four MLV systems in fluid phase

	DMPC	DPPC	POPC	DOPC
$E_a(f)$ ($kcal\ mol^{-1}$)	8.0	5.5	5.6	6.2
$E_a(K)$ ($kcal\ mol^{-1}$)	11.6	6.2	6.4	7.2

Values of E_a were obtained from Arrhenius plots of particular experiments. Errors in E_a estimated from the results of several independent experiments are estimated to be 20%.

relative magnitudes for both rates are shown to be in the order: DPPC > DMPC > POPC > DOPC. This means that in an environment of saturated lipid chains (DMPC, DPPC) both f and K are seen to increase with acyl chain length and this is consistent with the free volume model, because the volume per lipid acyl chain increases with chain length (Cohen and Turnbull, 1959; Vaz et al., 1985). Furthermore, Fig. 10 demonstrates that when the acyl chains contain *cis*-double bonds, the rates f and K decrease with the number of such kink-containing acyl chains, presumably because these chains impede the rotation of the pyrene moieties more effectively than fully saturated ones.

The relative magnitudes of K and f for the different probe-matrix systems follow the same order as the values of the available free areas of the four phospholipids in their bilayers: DPPC > DMPC > POPC > DOPC. This is again consistent with the free volume model for lateral diffusion as discussed by Vaz et al. (1985).

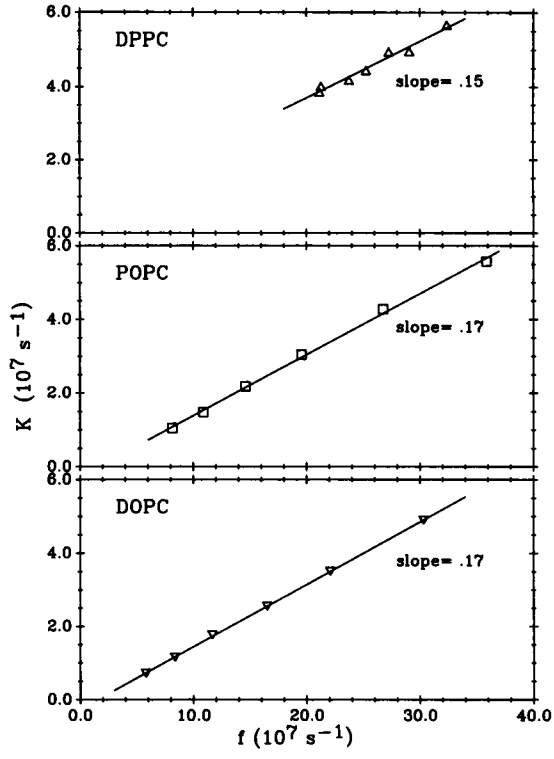

FIGURE 9 K, the excimer formation rate of $dipy_{10}$ PC in DPPC, POPC, and DOPC multilamellar vesicles in their fluid phase, plotted against f, the diffusion rate of py_{10} PC probes, measured at the same temperature. From the slopes of the straight lines here and in Fig. 8, one may obtain estimates of p_E (c.f. Eq. 19).

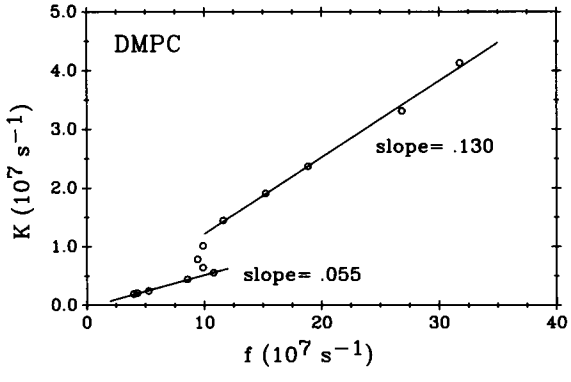

FIGURE 8 A plot of the intramolecular and intermolecular rates, K and f, determined for $dipy_{10}$ PC and py_{10} PC probes, respectively, in DMPC MLV at temperatures between 5 and 50°C. From the slopes of the linear portions below and above the transition temperature one obtains values of p_E by use of Eq. 18. DMPC is the only vesicle system for which Eq. 15 provides a satisfactory fit to the monopyrenyl probe titration data in the bilayer's gel, as well as in its fluid phase (c.f. Fig. 6). It is therefore the only system for which absolute values of f and K could be compared in both lipid phases.

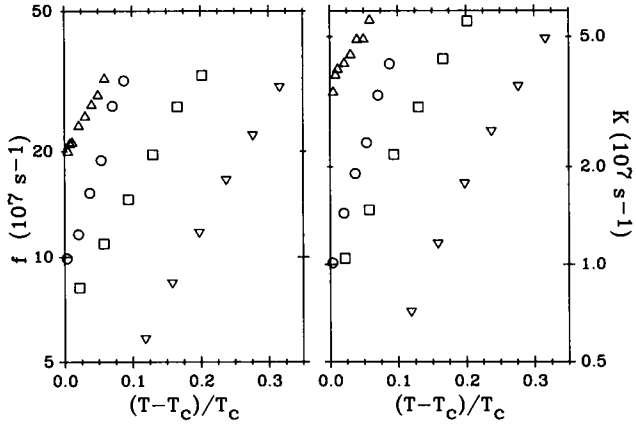

FIGURE 10 The rates K and f measured in four host bilayers in their fluid phase, plotted as a function of the reduced temperature, $(T - T_c)/T_c$, where T_c is the fluid-gel transition temperature.

The diffusion coefficients, D, for py_{10} PC in the four bilayers in their fluid phases have magnitudes which are comparable to the values obtained for long-range diffusion in FPR experiments, which include determinations for several fluorescent lipid analogues in DMPC, with D ranging from 9 to 18 $\mu m^2 s^{-1}$ at 38°C (Derzko and Jacobson, 1980) and from 5.5 to 8 $\mu m^2 s^{-1}$ at 30°C (Wu et al., 1977). These FPR studies in DMPC, including those in the gel phase, are discussed in greater detail in the companion paper (Sassaroli et al., 1990). FPR was also used to measure lipid analogue diffusivity in other fluid multibilayer systems, yielding D values of ~7 $\mu m^2 s^{-1}$ for DPPC at 45°C (Wu et al., 1977), and between 3 and 9 $\mu m^2 s^{-1}$ for POPC between 15 and 45°C (Vaz et al., 1985). While these values for the long-range diffusion coefficients are somewhat lower than those given in Table 1, they are of the same order of magnitude, given the uncertainties in analysis of both experimental techniques. The activation energies shown in Table 2 are comparable to values of E_a obtained in FPR experiments, which have been reported to range from 4 to 13 Kcal/mol for different probes in DMPC multibilayers (Derzko and Jacobson, 1980; Wu et al., 1977).

It is noted that the long-range diffusivity of lipid analogues in DMPC below T_c, is at least two orders of magnitude smaller than the local diffusivity measured by excimeric probes, as discussed in greater detail in the companion paper (Sassaroli et al., 1990).

Intramolecular excimeric lipid analogues are sensitive probes of membrane dynamics not only for model systems, like those studied here, but also for biological membranes: Preliminary studies in our laboratory have been shown that $dipy_{10}$ PC probes in erythrocyte membranes are sensitive to its cholesterol content and phospholipid composition. The magnitude of the cholesterol effect depends moreover dramatically on the length of the pyrenyl acyl chains of the probes, which suggests that different dipyrenyl PC probes sense membrane fluidity at different depths. Dipyrenyl probes are also, because of the large dynamic range and concentration independence of r_1, promising tools for determining the spatial distribution of membrane fluidity by use of spectrally resolved fluorescence microscopy.

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