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FLUORESCENCE QUENCHING IN MODEL MEMBRANES

AN ANALYSIS OF THE LOCAL PHOSPHOLIPID ENVIRONMENTS OF DIPHENYLHEXATRIENE AND GRAMICIDIN A'

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Interactions between the fluorophors diphenylhexatriene or gramicidin A' and lipids are examined using a spin-labeled phosphatidylcholine as a fluorescence quenching probe. It is found that in phospholipid vesicles of mixed lipid composition at temperatures where phospholipids are completely in the liquid crystal phase, several different species of phosphatidylcholines are randomly distributed around the fluorophors. In vesicles of mixed lipid composition which can undergo thermally induced phase separations, the fluorescence quenching observed at lower temperatures reflects a non-random distribution of lipids around each fluorophor. This observation is explained in terms of the partition of fluorophor between a spin-labeled lipid-rich liquid crystal phase, and a spin-labeled lipid-depleted gel phase. Gramicidin A' strongly favors partition into the liquid crystal phase, whereas diphenylhexatriene partitions about equally between the two lipid phases. A method is described utilizing fluorescence quenching for the calculation of the partition coefficient for a fluorophor. The partition coefficients so calculated are shown to be in good agreement with previously reported values derived from other methods. It is also shown that Ca^{2+}-induced lipid phase separations can be monitored by fluorescence quenching.

Introduction

The fluorescence quenching of a membrane-bound fluorophor by spin-labeled phospholipid can be used to detect contact between the fluorophor and the lipid [1], thereby enabling detailed examination of the composition of the local lipid environment around many fluorophors, including proteins. At temperatures where liquid crystal and gel phases coexist in mixtures of two or more lipids the lipid composition of each phase is different [2,3]. The distribution of any hydrophobic molecule between the two phases, and thus the type of lipids around that molecule, are determined by the partition coef-

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Abbreviations: DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DLPC, 1,2-dilauroyl-*sn*-glycero-3-phosphocholine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; egg PC, diacyl-*sn*-glycero-3-phosphocholine derived from chicken egg yolk; I(7,6) palmitic acid, 5-(6'-carboxyhexyl)-5-octyl-1-oxyl-2,2-dimethyloxazolidine; (7,6)PC, 1-acyl-2-I(7,6)-palmitoyl-*sn*-glycero-3-phosphocholine; (7,6)PA, 1-acyl-2-I(7,6)-palmitoyl-*sn*-glycero-3-phosphoric acid.

ficient of that molecule between liquid crystal and gel phases.

The partition behavior of molecules incorporated in real and model membranes has been studied by several methods. Partition of the small organic compounds diphenylhexatriene and perylene between liquid crystal and gel phases has been examined using the different fluorescence properties in each phase as a probe [4–6]. Partition behavior of the small polypeptide gramicidin A′ has been examined by differential scanning calorimetry [7]. Partition of membrane proteins has been examined by electron microscopy, including studies on glycophorin [8] and the Ca^{2+}-ATPase [9]. The temperature dependence of the activation energy of enzymatic function has been used as a probe of protein partition behavior [10] as well.

Fluorescence quenching by nitroxides has been applied previously to the study of lipid mixtures using perylene as a probe [11] and to the measurement of phospholipid binding to a membrane protein [12,13]. We have shown that fluorescence quenching of fluorophors by spin-labeled phospholipids occurs via a static mechanism in which only lipids in contact with the fluorophor can quench fluorescence [1]. This report demonstrates that fluorescence quenching of membrane-bound fluorophors responds both to thermally-induced and to Ca^{2+}-induced phase separations. A method is described for calculating the partition coefficients of fluorescent molecules between gel and liquid crystal phases, utilizing fluorescence quenching experiments. The partition coefficients obtained from fluorescence quenching are shown to be in good agreement with the values obtained by other methods, and thus the ability of fluorescence quenching to probe the lipid environment quantitatively is established.

Materials and Methods

DMPC (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine), was obtained from Sigma. Diphenylhexatriene was obtained from Aldrich. DPPC was obtained from Calbiochem. All other commercially obtained chemicals were reagent grade.

DOPC was synthesized by acylation of *sn*-glycero-3-phosphocholine with oleic anhydride [14] and purified by TLC in chloroform/methanol/water 65 : 25 : 4 (v/v). Egg PC was isolated from fresh egg yolks [15].

Spin-labeled phospholipid was prepared by condensation of I(7,6) palmitic acid with lyso egg PC as described previously [1,12]. The purity of 1,6-diphenyl-1,3,5-hexatriene and gramicidin A′ was verified with ^{1}H-NMR. The chemical shift and spin-spin splitting patterns matched those of standard spectra and no impurity resonances could be detected.

Fluorophors were incorporated in multilamellar phospholipid vesicles as follows. Stock solutions of gramicidin A′ in ethanol and diphenylhexatriene in chloroform were 0.1 mM. Fluorophor (0.8 nmol) was mixed with 125 nmol phospholipid dissolved in chloroform or ethanol. A few drops of chloroform were added and each sample was mixed vigorously. The samples were then dried under a flow of N_2 gas while warming gently (35–45°C). Samples were further dried under vacuum for 10 min. 2.5 ml water were added to each sample. The samples were then vigorously mixed above the phase transition temperature until a constant absorbance was reached of 0.05–0.1 at 360 nm (1-cm light path).

Fluorescence measurements were obtained using a Perkin-Elmer MPF-3 spectrofluorimeter. The excitation/emission wavelengths used were 290/340 nm for gramicidin A′ and 358/430 for diphenylhexatriene. Nominal excitation and emission bandwidths of 10 nm were used. The temperature dependence of fluorescence was measured in a thermostat-controlled cuvette holder and the temperature was measured with a digital thermometer (Markson Scientific) calibrated to ±1°C, placed in one sample cell in the cuvette holder. Samples were prepared at a temperature at which the phospholipids present were in the liquid crystal state, then the samples were cooled to 10–15°C and fluorescence was measured as temperature was increased slowly by up to approx. 1°C/min). Differential scanning calorimetry using a Perkin-Elmer DSC-2 was performed on samples of phospholipid swelled in the liquid crystal state with excess ethylene glycol/water (1/1, w/w).

Results

Fluorescence quenching is observed as a drop in fluorescence intensity in the presence of (7,6)PC

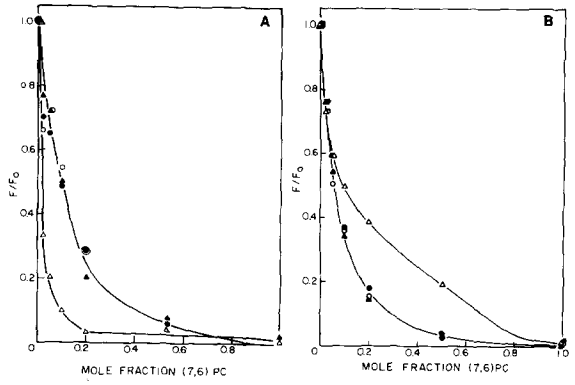

Fig. 1. Fluorescence quenching of gramicidin A′ and diphenylhexatriene incorporated into aqueous multilamellar liposomes containing (7,6)PC and an unlabeled PC species. The abscissa is the mole fraction of (7,6)PC in the lipids. The ordinate is the ratio of fluorescence in the presence (F) and absence (F_0) of (7,6)PC. See text for details of sample preparation. (A) (left) Gramicidin A′ incorporated into (7,6)PC/egg PC at 23°C (•), (7,6)/PC/DOPC at 23°C (○), (7,6)PC/DPPC at 23°C (△), (7,6)PC/DPPC at 44°C (▲). (B) (right) diphenylhexatriene incoporated into (7,6)PC/egg PC at 23°C (•), (7,6)PC/DOPC at 23°C (○), (7,6)PC/DPPC at 23°C (△), (7,6)PC/DPPC at 44°C (▲). The fluorophor to lipid ratio in these experiments and subsequent experiments was 1/156 (mol/mol).

relative to fluorescence intensity in the absence of (7,6)PC. The fluorescence quenching curves of diphenylhexatriene and gramicidin A′ incorporated into bilayer vesicles of (7,6)PC/unlabeled PC mixtures are shown in Fig. 1. The fluorescence quenching curves in Fig. 1A and B for (7,6)PC with egg PC, DOPC or DPPC (45°C) are nearly identical. In each of these cases the phospholipids are in the liquid crystal phase. The shape of these curves is close to that expected for the random distribution around the fluorophor of spin-labeled and unlabeled phospholipids, and it has been previously shown that these curves can be described approximately by $F/F_0 = (1 - [(7,6)PC]^6)$ [1]. In contrast, the curves of fluorescence quenching for gramicidin A′ and diphenylhexatriene in (7,6)PC/DPPC at 23°C are different from the others. For gramicidin A′ in (7,6)-PC/DPPC at 23°C quenching is significantly enhanced relative to the other curves in Fig. 1A. For diphenylhexatriene in (7,6)PC/DPPC at 23°C quenching is significantly reduced relative to the other curves in Fig. 1B, except at very low concentrations of (7,6)PC (<0.05 mole fraction).

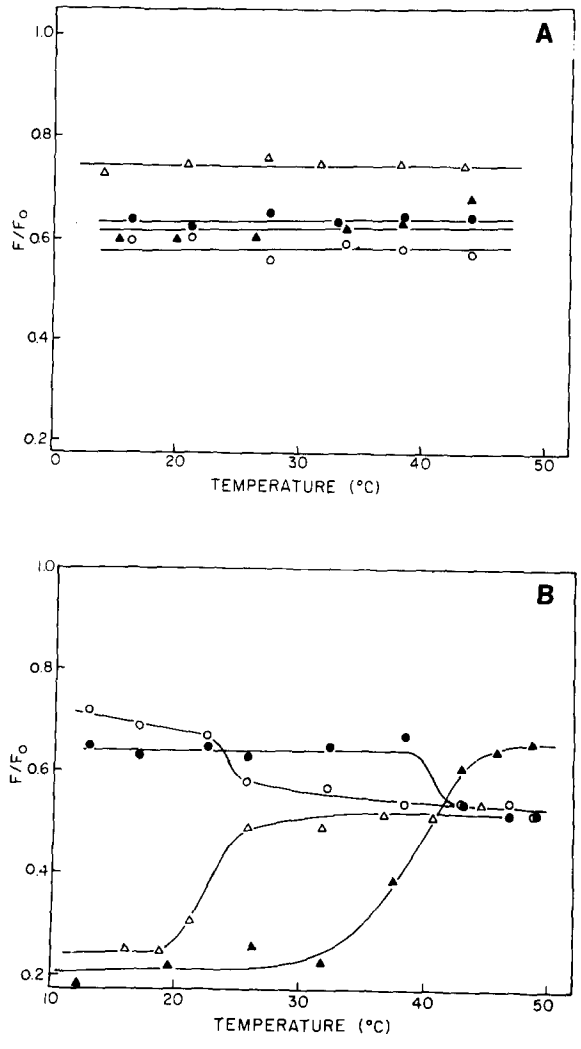

Fig. 2. Temperature dependence of fluorescence quenching of gramicidin A′ and diphenylhexatriene in multilamellar liposomes of PC containing 0.05 mole fraction (7,6)PC. (A) (top) diphenylhexatriene incorporated into (7,6)PC/egg PC (•), (7,6)PC/DOPC (○); gramicidin A′ incoporated into (7,6)PC/egg PC (▲), (7,6)PC/DOPC (△). (B) (bottom) diphenylhexatriene incorporated into (7,6)PC/DMPC (○), (7,6)PC/DPPC (•); gramicidin A′ incorporated into (7,6)PC/DMPC (△) and (7,6)PC/DPPC (▲).

The relationship of these effects to the liquid-crystal-to-gel phase transition of lipids is seen more clearly in Fig. 2. Here, the temperature dependence of fluorescence quenching for diphenylhexatriene and gramicidin A′ in vesicles of 0.05 mole fraction (7,6)PC is shown. In Fig. 2A (7,6)PC/egg PC and

(7,6)PC/DOPC vesicles show temperature-independent fluorescence quenching, as is expected for a static fluorescence quenching process [1]. In these experiments the liquid crystal-to-gel phase transition of egg PC, DOPC and (7,6)PC occur below the lowest temperatures illustrated (see below), and thus these phospholipid mixtures are in the liquid crystal state.

In Fig. 2B a sharp break is seen in the fluorescence quenching vs. temperature curves for both diphenylhexatriene and gramicidin A'. In 0.05 mole fraction (7,6)PC/DMPC the break occurs at about 24°C and in 0.05 mole fraction (7,6)PC/DPPC the break occurs at about 40°C. These temperatures correspond to the liquid-crystal-to-gel phase transitions of DMPC and DPPC, respectively. Below the nominal phase-transition temperatures fluorescence quenching of diphenylhexatriene is reduced but fluorescence quenching of gramicidin A' is increased. Thus it appears that the rearrangement of phospholipid structure associated with the phase transition of DMPC or DPPC results in a change in the type of phospholipid around the fluorophor, although diphenylhexatriene and gramicidin A' respond differently to phase separation.

Further analysis of these fluorescence-quenching results requires knowing the composition and amount of the lipid phases as a function of temperature. The partial phase diagram for (7,6)PC/DPPC is shown in Fig. 3. The phase diagram is intermediate between the diagrams for DMPC/DSPC and DLPC/DSPC, in which there is partial co-crystallization or almost complete lack of co-crystallization, respectively [16]. These mixtures and (7,6)PC/DPPC all exhibit a two-phase region in which gel and liquid crystal phases of very different composition exist in equilibrium. In the two-phase region for (7,6)PC/DPPC the gel state is nearly pure DPPC with only a few percent of (7,6)-PC. The liquid crystal state is enriched in (7,6)PC and below 35°C is primarily (7,6)PC. We were unable to determine the liquid crystal-to-gel phase-transition temperature of pure (7,6)PC by differential scanning calorimetry, perhaps because the phase transition occurs over a wide temperature range due to acyl chain heterogeneity [1], and only the high temperature limit of the phase transitions could be observed. Osmotic swelling and NMR studies indicate that (7,6)PC remains in the liquid crystal state down to at least 12°C [1]. A small region of co-crystallization of

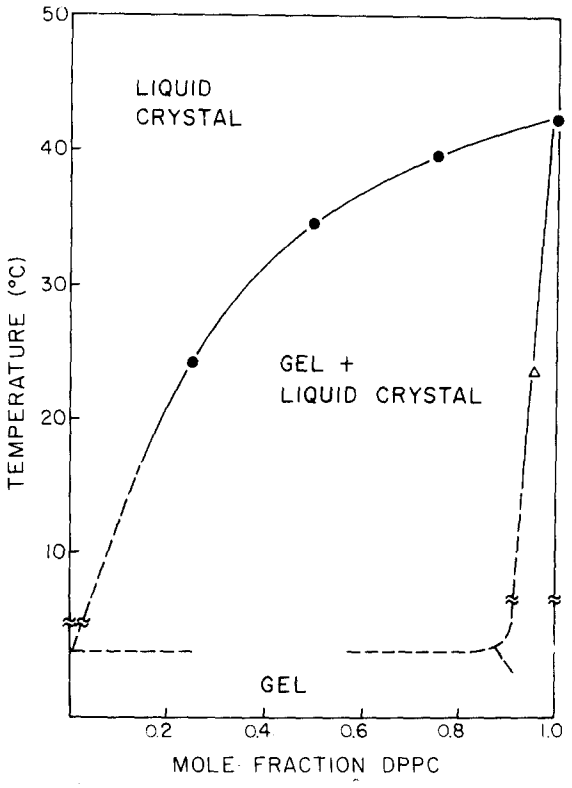

Fig. 3. Partial phase diagram for multilamellar membranes of (7,6)PC/DPPC. (•) Points determined by differential scanning calorimetry. (△) Point determined from fluorescence quenching of diphenylhexatriene. Dashed lines are hypothetical. See text for details.

a presumably homogenous gel phase exists to the right of the solidus line, and is expected to be present in mixtures of a minute amount (7,6)PC in DPPC. The gel phase at very low temperature has not been observed in these studies but might consist of two gel phases, one a virtually pure (7,6)PC phase and a separate, nearly pure, DPPC phase. Such a lack of co-crystallization has been observed in several binary phospholipid mixtures where the phase-transition temperatures of the two phospholipids are very different, including cases in which the fatty acyl chain lengths are similar [2].

Using the phase diagram, the fluorescence-quenching data can now be understood. The abrupt change in fluorescence quenching in 0.05 mole fraction (7,6)PC/DPPC at 40°C represents the temperature at which there is a transition between the one-phase liquid crystal system and coexisting gel and liquid

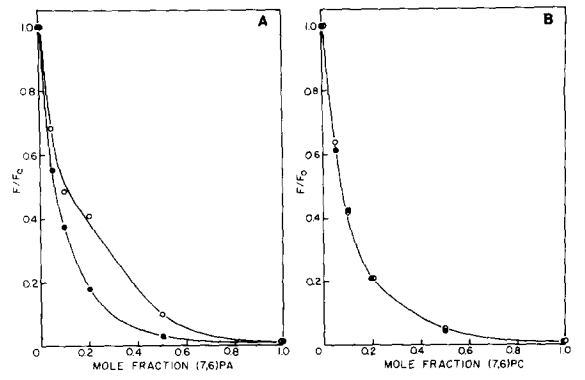

Fig. 4. Effect of the presence of Ca^{2+} upon fluorescence quenching of diphenylhexatriene incorporated into multilamellar membranes containing egg PC and spin-labeled phospholipid dispersed in 20 mM Tris-HCl, pH 7.5, 23°C. (A) (left) diphenylhexatriene incorporated in (7,6)PA/egg PC in the absence (•) and presence (○) of 5 mM $CaCl_2$. (B) (right) diphenylhexatriene incorporated into (7,6)PC/egg PC in the absence (•) and presence (○) of 5 mM $CaCl_2$.

crystal phases. This occurs close to the transition temperature of pure DPPC. Below 40°C the fluorescence reflects the distribution of the fluorophor between the (7,6)PC-depleted gel and the (7,6)PC-rich liquid crystal phase. Thus Figs. 1 and 2 clearly illustrate that gramicidin A′, in contrast to diphenylhexatriene, prefers the liquid crystal, (7,6)PC-rich phase.

Ca^{2+}-induced phase separation can also be detected by fluorescence quenching as shown in Fig. 4. In the absence of Ca^{2+}, fluorescence quenching of diphenylhexatriene by (7,6)PA and (7,6)PC is about the same. To test the effect of Ca^{2+}, multilamellar liposomes were prepared in Ca^{2+}-containing buffer. In the presence of 5 mM Ca^{2+}, fluorescence quenching in (7,6)-PA/egg PC is seen to be markedly reduced. The Ca^{2+}-induced change in the fluorescence quenching curve in (7,6)PA/egg PC resembles that observed upon thermally induced phase separation of (7,6)PC/DPPC and indicates that there has been separation into spin-label-rich and spin-label-depleted phases. The fluorescence quenching of diphenylhexatriene is not affected by Ca^{2+} in model membranes of (7,6)-PC/egg PC, showing that direct Ca^{2+}-DPH interaction is not involved. The phase separation seems to occur with as little as 0.05 mole fraction (7,6)PA in the multilamellar vesicles. We have observed related effects of phase separations upon the fluorescence quenching of the membrane-bound Ca^{2+}-ATPase enzyme in (7,6)PC and (7,6)PA [12].

Discussion

The response of fluorescence quenching to changes in the phospholipid environment of fluorescent molecules incorporated into model membranes containing spin-labeled phospholipids is examined in this study. An increase in the concentration of spin-labeled phospholipids in contact with a fluorophor results in an increase in fluorescence quenching, whereas a decrease in this local concentration of spin-labeled phospholipids results in a decrease in fluorescence quenching. When the membranes are in the fluid liquid crystal state, the superimposability of the fluorescence quenching curves for various species of PC indicates that these phospholipid species randomly distribute around both a small rigid hydrocarbon, diphenylhexatriene, and a hydrophobic polypeptide, gramicidin A′. It also appears as if (7,6)PC and (7,6)PA are distributed around diphenylhexatriene in a random manner in the absence of Ca^{2+}.

There have been proposals, based mainly on theoretical considerations, that there should be some clustering of phospholipids in the liquid crystal state [17,18]. Fluorescence quenching does not detect any preferential clustering around the fluorophors by the phospholipids used in this study. This result is not surprising since specific binding of lipids to diphenylhexatriene and gramicidin A′, which are both small uncharged molecules, would not be expected. Recent crosslinking studies also fail to detect clustering or specific associations in the liquid crystal phase [19].

The experiments reported here show that for model membranes consisting of (7,6)PC/DMPC or (7,6)PC/DPPC, fluorescence quenching is temperature dependent, and at lower temperatures the phospholipid environment of the fluorophors drastically changes. The change in fluorescence quenching of diphenylhexatriene and gramicidin A′ reflects the liquid-crystal-to-gel phase transition, with resulting phase separation of a (7,6)PC-rich liquid crystal and (7,6)PC-depleted gel phase. The fluorescence quenching observed at temperatures at which the two phases coexist can be discussed in terms of partitioning of

fluorophor between the two phases. If a substance partitions strongly into the spin-label-rich liquid crystal phase then there will be more fluorescence quenching than at a temperature at which a single, homogeneous phase is present, for the same total concentration of spin label. If a substance partitions strongly into the spin-label-depleted gel phase then it will be quenched less than in the single homogeneous phase. Qualitatively similar behavior has been previously observed for spin-labeled cholestane and perylene. Below the liquid-crystal-to-gel phase transition temperature, in a matrix of DPPC, clustering of spin label and fluorophor was observed [11].

Using the fluorescence-quenching data together with the phase diagram for the (7,6)PC/DPPC multilamellar vesicles, the partition coefficient between liquid crystal and gel phases can be calculated. The partition coefficient of a molecule A between liquid crystal and gel phases is defined as:

$$K_p = \frac{\text{Volume of gel phase}}{\text{Volume of liquid crystal phase}} \times \frac{\text{Amount of A in liquid crystal phase}}{\text{Amount of A in gel phase}} \quad (1)$$

So defined, $K_p > 1$ corresponds to preferential partition into a liquid crystal phase. Derivation of the partition coefficient from the experimental data is described in the Appendix.

Once the binary lipid phase diagram and the dependence of quenching upon [(7,6)PC] in a homogeneous phase are both known, then the fluorescence quenching observed in a two-phase region will depend solely on K_p. Theoretical fluorescence quenching curves for various partition coefficients are shown in Fig. 5. Notice that quenching is linear in [(7,6)PC] for $K_p = 1$.

From data in Fig. 1, the value of K_p calculated for diphenylhexatriene in membranes of (7,6)PC/DPPC is 1–2. This indicates that diphenylhexatriene does not greatly prefer to be in either the liquid crystal or gel state. This value of K_p is in good agreement with the value of 1 found previously [4] for the partition of diphenylhexatriene between liquid crystal DMPC vesicles and gel phase DPPC vesicles. Partition coefficients close to unity have also been reported for perylene, another small organic fluorophor [5] and *cis*-paranaric acid [6].

A value of $K_p \approx 100$ is observed for gramicidin.

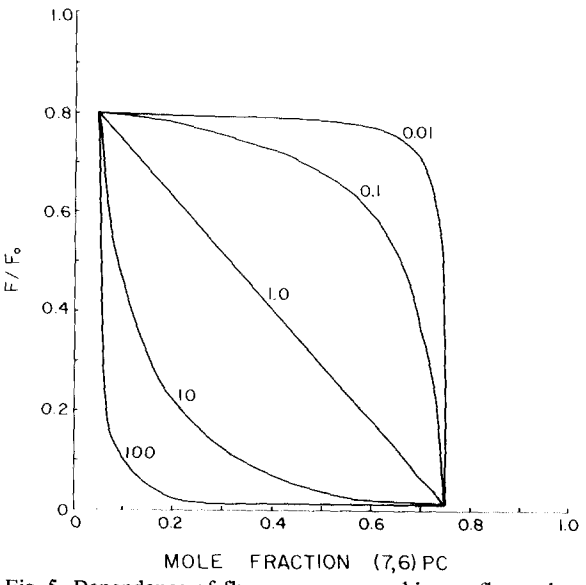

Fig. 5. Dependence of fluorescence quenching on fluorophor partitioning between coexisting liquid crystal and gel phases. K_p, the partition coefficient, is indicated next to each curve, larger values reflecting increasing partition into the liquid crystal phase. The curves are calculated for a mole fraction of (7,6)PC in the gel phase of 0.05 with $(F/F_0)_G = 0.8$, and a mole fraction of (7,6)PC in the liquid crystal phase of 0.75 with $(F/F_0)_{LC} = 0.01$. The curve which describes the fluorescence quenching outside the two phase region is not shown.

This shows the marked preference of this polypeptide for the liquid crystal phase over the gel phase when both phases are present. This is in agreement with calorimetric studies of the partitioning of gramicidin A′ in a DLPC/DPPC system [7]. It was found that gramicidin A′ partitioned favorably into the DLPC-rich liquid crystal phase, although no value of K_p could be calculated. The fact that gramicidin A′ does not alter the transition temperature of pure DPPC, when scanned from low to high temperatures, is consistent with insolubility of gramicidin A′ in the gel phase [7,20].

Qualitatively the fluorescence-quenching results for gramicidin A′ in (7,6)PC/DMPC or (7,6)PC/DPPC resemble those for the enzyme Ca^{2+}-ATPase [12]. This is consistent with the preference of Ca^{2+}-ATPase for the liquid crystal state, as has been observed by Kleeman and McConnell [9]. Thus fluorescence-quenching results for these three fluorescent molecules are consistent with the results obtained by other methods.

Many factors might influence the differences in the partition behavior of gramicidin A′ and diphenylhexatriene. Disruption of the ordered structure of the gel phase by a polypeptide or protein could be a critical determinant of partition behavior. On the other hand, a small number of diphenylhexatriene molecules might be able to enter the gel or liquid crystal phase without disturbing the phospholipid lattice, and thus partition equally between gel and liquid crystal phases. Previous studies have demonstrated that phospholipid vesicles can bind a limited number of diphenylhexatriene molecules before the vesicles are saturated [22].

Another important aspect of fluorescence quenching and temperature-induced phase separation concerns the sensitivity of fluorescence quenching to the phase behavior of model membranes containing only a few percent (7,6)PC. At less than 0.05 mole fraction (7,6)PC in DPPC the fluorescence quenching of diphenylhexatriene is the same at 23°C and at 45°C. This is reasonable if there exists a single homogeneous gel phase of a few per cent (7,6)PC at 23°C as well as the expected homogeneous liquid crystal phase at 45°C, and if fluorescence quenching is the same in a homogeneous gel phase as in a homogeneous liquid crystal phase. The mole fraction of (7,6)-PC at which the fluorescence quenching at 23°C diverges from that at 45°C is the point at which lipid phase separation appears at 23°C. This fact was used in the construction of the partial phase diagram in Fig. 3. By varying the temperature an entire phase diagram could in principle be obtained solely from fluorescence-quenching data. The narrow region of homogeneous gel phase (co-crystallization) detected in (7,6)PC/DPPC by fluorescence quenching should be postulated for binary mixtures of phospholipids, such as DLPC/DSPC, which are immiscible throughout most of the gel state [16]. However, this region has not been detected previously because it is difficult to measure accurately the solidus line in this area of the phase diagram using calorimetry.

Fluorescence quenching of gramicidin A′ seems to detect phase separation at 0.025 mole fraction (7,6)-PC in DPPC. In view of the extreme insolubility of gramicidin A′ in a gel phase it seems likely that at very low concentrations of (7,6)PC, gramicidin A′ is perturbing the phase behavior of the phospholipids and inducing phase separation of a (7,6)PC-rich phase at a lipid composition where such separation would not ordinarily occur. This can be a significant source of error if partition behavior is only examined at very low mole fractions of (7,6)PC. Another possible source of error is specific binding of either (7,6)PC or an unlabeled lipid. Although we have not observed specific binding in the liquid crystal state, specific binding might occur in the gel state. Another possible problem, differential fluorescence quenching by oxygen in liquid crystal and gel phases, is not likely to be significant since oxygen quenching of diphenylhexatriene is very weak (less than 5%) at atmospheric pressure [23] and should be even weaker for the short-lived excited states of the tryptophanyl residues of gramicidin A′.

Ca^{2+}-induced as well as temperature-induced phase separations are detected by fluorescence quenching. ESR studies have also detected Ca^{2+}-induced phase separations in spin-labeled PA/PC model membranes [24,25]. Such phase separations in model membranes with Ca^{2+}-ATPase as the fluorophor are discussed in detail elsewhere [12]. It is noteworthy that in principle phase separations would be detected by fluorescence quenching of diphenylhexatriene even if both phases are in the liquid crystal state.

In summary, fluorescence quenching can be used to calculate the partition coefficient of fluorescent molecules between a liquid crystal and a gel phase. The partition coefficients so obtained are in good agreement with those obtained by other methods. The methods described here, unlike others, do not require a difference in fluorophor properties in gel and liquid crystal states. More importantly, these experiments show that fluorescence quenching accurately reflects the phospholipid environment of a fluorophor. Fluorescence quenching is not limited to measurements at phase transitions and this allows application of fluorescence quenching to the determination of the binding strenght of different lipids to membrane proteins, as described elsewhere [1,12,13].

Appendix

Calculation of partition coefficients using fluorescence quenching

Consider the case of a fluorophor incorporated into model membranes with two lipid phases, one

rich in (7,6)PC and one depleted in (7,6)PC, relative to some overall mole fraction of (7,6)PC in the membranes. To calculate the partition coefficient the amount of fluorophor in each phase must first be determined. The observed fluorescence in the two-phase system is given by

$$(F/F_0)_{obs} = (\text{fluorophor})_{LC}(F/F_0)_{LC} + (\text{fluorophor})_{G}(F/F_0)_{G} \quad (A.1)$$

where:

$(F/F_0)_{obs}$ = observed fluorescence intensity in the membranes containing (7,6)PC relative to the fluorescence intensity in the absence of (7,6)PC

$(\text{fluorophor})_{G(LC)}$ = fraction of all fluorophor molecules in the gel (liquid crystal) phase

$(F/F_0)_{G(LC)}$ = fractional fluorescence quenching of a fluorophor in the gel (liquid crystal) phase region of the model membranes.

The fractional fluorescence quenching in each phase is given by:

$$(F/F_0)_G = f([(7,6)PC]_G) \quad (A.2)$$

$$(F/F_0)_{LC} = f([(7,6)PC]_{LC}) \quad (A.3)$$

where:

$[(7,6)PC]_{G(LC)}$ = mole fraction of gel (liquid crystal) phase that is composed of (7,6)PC

$f(\ )$ = function describing the shape of the curve of fluorescence quenching vs. mole fraction (7,6)PC within each phase.

$[(7,6)PC]_G$ and $[(7,6)PC]_{LC}$ are obtained from the phase diagram (Fig. 3). These compositions are given by the intercepts of the experimental temperature (tie) line with the liquidus and solidus lines. Although an approximate analytical expression for the function f is described by $1 - [(7,6)PC]^6$ [1], the experimental curves are used for calculation of the partition coefficient. With diphenylhexatriene, for example, the f function is described by the fluorescence quenching curve shown in Fig. 1 for mixtures of (7,6)PC/DOPC, (7,6)PC/egg PC and, at 45°C, (7,6)-PC/DPPC. The appropriate $(F/F_0)_G$ and $(F/F_0)_{LC}$ values for the mole fractions of (7,6)PC in each phase can be read off the Y-axis of the graph. It is assumed that f is the same function in a homogeneous gel state and in a homogeneous liquid crystal state (see Discussion). The values of $(F/F_0)_G$ and $(F/F_0)_{LC}$ obtained above are substituted into Eqn. A.1. To solve for the fraction of total fluorophor in each phase the Eqn. A.1 is now combined with

$$(\text{fluorophor})_{LC} + (\text{fluorophor})_{G} = 1 \quad (A.4)$$

The partition coefficient is given by:

$$K_p = \frac{[G]\,\rho_{LC}(\text{fluorophor})_{LC}}{[LC]\,\rho_G(\text{fluorophor})_G} \quad (A.5)$$

where:

$\frac{[G]}{[LC]}$ = the mole ratio of lipids in the gel phase to lipids in the liquid crystal phase, and

$\frac{\rho_{LC}}{\rho_G}$ = the density ratio of the two phases.

Bilayer studies show that ρ_{LC}/ρ_G is approximately unity [25,26]. The ratio [G]/[LC] is obtained directly from the phase diagram (Fig. 3) using the lever arm rule:

$$\frac{[G]}{[LC]} = \frac{[(7,6)PC] - [(7,6)PC]_{LC}}{[(7,6)PC]_G - [(7,6)PC]} \quad (A.6)$$

where [(7,6)PC] is the overall mole fraction of (7,6)-PC in the membrane. The theoretical curves of Fig. 5 were obtained by combining equations (A.1–6) to obtain:

$$\left(\frac{F}{F_0}\right)_{obs} = \left(\frac{F}{F_0}\right)_{LC} + \left[\left(\frac{F}{F_0}\right)_G - \left(\frac{F}{F_0}\right)_{LC}\right] \times \frac{[G]}{K_p - K_p[G] + [G]} \quad (A.7)$$

where:

$$[G] = \frac{[(7,6)PC]_{LC} - [(7,6)PC]}{[(7,6)PC]_{LC} - [(7,6)PC]_G}$$

K_p can now be estimated by fitting the experimental data to the theoretical curves.

In this derivation it has been assumed that there is homogeneous distribution of phospholipid within each phase. It has also been assumed that in the sample in which fluorescence quenching has been measured the presence of fluorophor does not significantly perturb the phase behavior of the phospholipids (see Discussion). A correction must be made in either Eqn. A.2 or A.3 if the quantum yield of fluorescence in the absence of (7,6)PC is not the same in a gel and liquid crystal phase. For example, if the fluorescence in the absence of spin-label, F_0, is measured for fluorophor in a gel state phospholipid then a correction would be required in Eqn. A.3 because fluorescence originating from the liquid crystal region formed when (7,6)PC is present is reduced by quenching and is affected by the change in quantum yield in a liquid crystal state relative to a gel state. In this case:

$$(F/F_0)_{LC_{corrected}} = f([7,6)PC]_{LC} \cdot (F_0)_G/(F_0)_{LC} \qquad (A.8)$$

The ratio of the quantum efficiency of a fluorophor in the gel relative to that in the liquid crystal, $(F_0)_G/(F_0)_{LC}$, can be determined from the break in quantum efficiency at the liquid crystal-to-gel transition [28]. For gramicidin A′ and diphenylhexatriene the quantum efficiency in the liquid crystal seems to be no more than 1.5-times that in the gel state (unpublished observations). Because so little fluorescence arises from the liquid crystal phase, which is 0.75 mole fraction (7,6)PC at 23°C (see Fig. 3), the effect of the correction on the calculated K_p values in this report is small.

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