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Partitioning behavior of indocarbocyanine probes between coexisting gel and fluid phases in model membranes

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Gel-fluid partition coefficients, K_p, were measured for a series of indocarbocyanine dyes in multilamellar lipid vesicles. The dyes examined had alkyl chain lengths from 12 to 22 carbons. Fluorescence quenching by a spin-labeled phosphatidylcholine-enriched fluid phase created a large difference in quantum yield for indocarbocyanine fluorescence between fluid and gel phases, enabling reliable K_p determinations. The values range from $K_p = 8$ for the 12-carbon chain, favoring a fluid phase over a Ca^{2+}-phosphatidylserine rigid phase, to $K_p = 0.02$ for the 20-carbon chain dye, favoring a distearoylphosphatidylcholine-rich gel phase over the fluid phase.

Introduction

The indocarbocyanine dyes are a class of cationic lipophilic fluorescent probes that have been used extensively in the study of membrane properties. They have large molar absorptivities, acceptable quantum yields for fluorescence, and reasonable photostability [1]. Using fluorescence recovery after photobleaching, lateral diffusion of indocarbocyanine probes, C_nDiI *, has been studied in biological membranes [2–8] and in model vesicles [9–11]. In these latter studies it was found that the diffusion rate of the C_nDiI probes is independent of the alkyl chain length of the probe for fluid phase lipid, but that in lipid systems with both gel and fluid phases coexisting, the diffusion rate was dependent on the alkyl chain length. At least part of this dependence could be explained by the selective partitioning of the probe between gel and fluid phases [10]. Using fluorescence intensity [10] and differential scanning calorimetry [11], the shift in T_m of the thermal transitions of phosphatidylcholines (PC) was determined. It was found that an approximate match of the probe alkyl chain length with that of the PC acyl chains led to preferential partitioning of the probe into the gel phase, as indicated by an increase in T_m for the lipid.

Gel-fluid phase coexistence has been widely studied in model as well as real biomembranes [12–24,37]. In order to understand molecular-level details of motion and location in membranes, it is necessary but not sufficient to detect gel-fluid coexistence. One method for obtaining quantitative information about details of gel-fluid properties is to determine the partition coefficient, K_p for the distribution of lipidic probes between the two phases. K_p is defined as the ratio of probe concentration in the fluid phase to that in the gel phase. Thus, $K_p > 1$ implies preference of the probe for fluid phase, and $K_p < 1$ preference for gel phase. The K_p values are characteristic of the probe in a particular gel-fluid mIxture, are well-defined and have predictive value [12–19]. K_p for fluorescent probe molecules can be determined by a method developed by London and Feigenson [20] in which the probe fluorescence in a spin

* C_nDiI used here refers to the 1,1′-dialkyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate, where n is the length of the alkyl chains.

Abbreviations: 16-AP, 16-(9-anthroyloxy)palmitic acid; DOPS, 1,2-dioleoyl-*sn*-glycero-3-phosphoserine; DPH, 1,6-diphenyl-1,3,5-hexatriene; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; EPR, electron paramagnetic resonance; PC, diacyl-*sn*-glycero-3-phosphocholine; (7,6)PC, 1-acyl-2-[2-(6-carboxyhexyl)-2-octyl-4,4-dimethyloxazolidinyl-3-oxy]*sn*-glycero-3-phosphocholine; Pipes, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; PS, diacyl-*sn*-glycero-3-phosphoserine; F, measured fluorescence intensity; F_0, measured fluorescence intensity in the pure gel phase; K_p, gel-fluid partition coefficient, defined as the ratio of probe concentration in the fluid phase to that in the gel phase.

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label-rich fluid phase, (7,6)PC, is quenched by contact with the labelled lipid. Various gel phases are formed with the (7,6)PC and the K_p value is determined from the difference in fluorescence of the probe caused by the difference in spin label-induced quenching in the gel and fluid phases.

Using this quenching method, Huang et at. [19] studied the distribution between (7,6)PC spin-labelled fluid phase and several gel phases, of anthroyloxy-fatty acids with the fluorescent moiety located at different positions along the fatty acyl chain. It was found that for gel phases of dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine (DSPC) there is little dependence of the partition coefficient on the position of the anthroyloxy moiety along the chain, with values of K_p between 4 and 5 (favoring the fluid phase) for DPPC and DSPC. The only probe that showed a significant difference was the 16-(9-anthroyloxy)palmitic acid, 16-AP, with the fluorescent group on the end of the fatty acyl chain, for which K_p is 1.5. Since the values of K_p show probe preference for the fluid phase lipid, we infer that the probe molecules are disruptive of the gel phase packing. The 16-AP, with the bulky group at the end of the fatty acyl chain, appears to be least disruptive of the gel phase. Fluorescence quenching measurements of these same anthroyloxy-labelled acids in the presence of coexisting $Ca(PS)_2$ gel and (7,6)PC-rich fluid phase show even stronger partitioning of the probes into the fluid phase, with K_p values around 18 for all of the series except for the 16-AP, which again showed less pronounced fluid phase preference ($K_p = 11$). These studies and others [21–24] on $Ca(PS)_2$ indicate the unique character of this gel phase. The binding of calcium ion to the negatively charged headgroups leads to very rigid, ordered acyl chains that tend to exclude other molecules from the gel. There are relatively few probes that have been found to partition significantly into the $Ca(PS)_2$ gel phase. Partition coefficients of 1 and 3 have been found for the 12-(9-anthroyloxy)-phosphatidylserine and *trans*-paranaric acid, respectively, in $Ca(PS)_2$/(7,6)PC mixtures [18].

In order to obtain more sensitivity to the differences among various kinds of gel phases, probe molecules are needed that preferentially partition into the gel phase. This paper is concerned with the partitioning behavior of the C_nDiI series of fluorescent probe molecules, some of which prefer gel-like environments, as mentioned above. In this study, we examine the partitioning behavior of C_nDiI probes in several gel-fluid phase systems using the fluorescence quenching technique of London and Feigenson [20]. This method has the advantage that partition coefficients are evaluated at very low probe concentrations so that there is little concentration-induced perturbation of the lipid. A common fluid phase, (7,6)PC, is mixed with three different gel phases, DPPC, DSPC and $Ca(PS)_2$. The DSPC phase is examined at two different temperatures. The partition coefficients of the C_nDiI probes within $n = 12$–22 are evaluated in the lipid mixtures, and the results are interpreted in terms of the interactions of the probe with the gel phase, and how those interactions reflect inherent properties of the gel. We find a variation in K_p of over two orders of magnitude, depending on the relative lengths of the probe alkyl chains and the PC acyl chains. The variation of K_p is greatest in DSPC, less in DPPC, and less yet in $Ca(PS)_2$ gel phase. The results presented here have implications for using the C_nDiI probes in fluorescence microscopy, and particularly for the detection of large gel regions in membranes.

Materials and Methods

The phospholipids DSPC, DPPC and DOPS, were obtained from Avanti Polar Lipids (Birmingham, AL), and were used without further purification. The (7,6)PC spin-labelled phospholipid was synthesized as described previously [20]. All of the indocarbocyanine dyes were purchased from Molecular Probes, Inc. (Eugene, OR). Fig. 1 shows the structure of the C_nDiI probes, with $n = 12$, 16, 18, 20, or 22 carbon alkyl groups on the fluorophores in our studies. Probe and lipid purity were checked by thin-layer chromatography with 20–50 μg samples on Absorbosil Plus P plates (Applied Science, State College, PA) using $CHCl_3$/MeOH/H_2O (65 : 30 : 5, v/v) or $CHCl_3$/MeOH/conc. NH_3 (64 : 30 : 6, v/v) as eluting solvents.

Samples for fluorescence measurements were prepared from lipid stock solutions in $CHCl_3$ and C_nDiI stock solutions in MeOH or $CHCl_3$ by mixing the appropriate volumes of stocks in 10 × 75 mm borosilicate culture tubes, evaporating the solvent to a thin film under argon and placing the tubes in vacuo overnight. Samples of DSPC/(7,6)PC or DPPC/(7,6)PC were then prepared by adding buffer (100 mM KCl, 20 mM Pipes at pH 7.0) to the thin films, sealing under argon, then heating to above the transition temperatures of the lipids (45 °C for DPPC and 58 °C for DSPC) for about 45 min. After mild vortexing the samples were returned to the heating blocks above the transition temperature for an additional 15 min and then allowed to cool to room temperature at a rate of about 0.5 deg/min. The lipid concentration in each 0.5 ml sample was 250 μM with the probe at a 1/20000 ratio to lipid. The con-

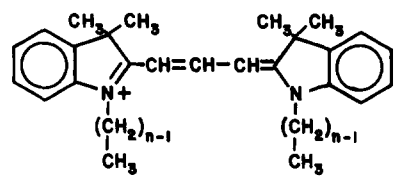

Fig. 1. Structure of the C_nDiI probes.

centrations of the C_nDiI stock solutions in MeOH were determined spectrophotometrically using the molar absorptivity according to Sims et al. [1].

In the case of the DOPS/(7,6)PC mixtures with and without Ca^{2+}, samples of 1 mM total lipid alter dilution with buffer and 1/20000 C_nDiI probe were prepared as above to obtain the thin films. The films were hydrated without Ca^{2+} by heating for 45 min at 40°C and vortexing. For the samples to be equilibrated with Ca^{2+}, additional buffer was then added to obtain a final aqueous composition of 100 mM KCl, 20 mM Pipes and 2 mM Ca^{2+} at pH 7.0. The Ca^{2+}-containing samples, 0.15 ml of 1 mM total lipid, were then subjected to 15 freeze-thaw cycles to equilibrate the $Ca(PS)_2$ system, and also to produce a fine dispersion of the lipids, as described previously [18]. Prior to the fluorescence measurements the samples were diluted with buffer containing 2 mM Ca^{2+} to a final lipid concentration of 100 μM in 1.5 ml. Dilutions were carried out directly into the acrylic cells used in fluorescence measurements. Because the transfer efficiency from the glass tubes was not 100% for the mixtures containing the larger amounts of $Ca(PS)_2$, the total phosphorous content of the samples in the fluorescence cells was determined for each sample as in Kingsley and Feigenson [25]. The fluorescence readings were then corrected for the efficiency of transfer [18].

Fluorescence measurements at 21°C were made with a home-built fluorimeter [26]. For the measurements at 35°C a Perkin-Elmer 650-40 spectrofluorimeter equipped with a water-jacketed cell holder was used, with the cell temperature determined with a calibrated thermister probe. Excitation was 540 nm, emission at 580 nm [1]. Emission spectra were run for samples in the pure gel and fluid phases to check for any spectral shifts due to the particular phases involved. Fluorescence of lipid samples without probes at the excitation and emission wavelengths used was insignificant compared with the sample fluorescence.

For examination of the nature of the gel phase in DPPC and DSPC mixtures with (7,6)PC, EPR spectra were obtained on 0.2 to 20 mole percent (7,6)PC, compositions in the single phase gel region. Samples were prepared from stock $CHCl_3$ solutions of the two components at 1 mM total lipid concentration (10 mM for the 0.2 mole percent solutions) in buffer, and were hydrated as described above for the fluorescence measurements. Spectra were recorded on a Varian E-4 X-band spectrometer with 100 kHz field modulation. Nine spectra were digitized and averaged with a DEC PDP 11/23 computer.

Results

Figs. 2–5 show the fluorescence quenching measurements obtained for the C_nDiI probes in mixtures of

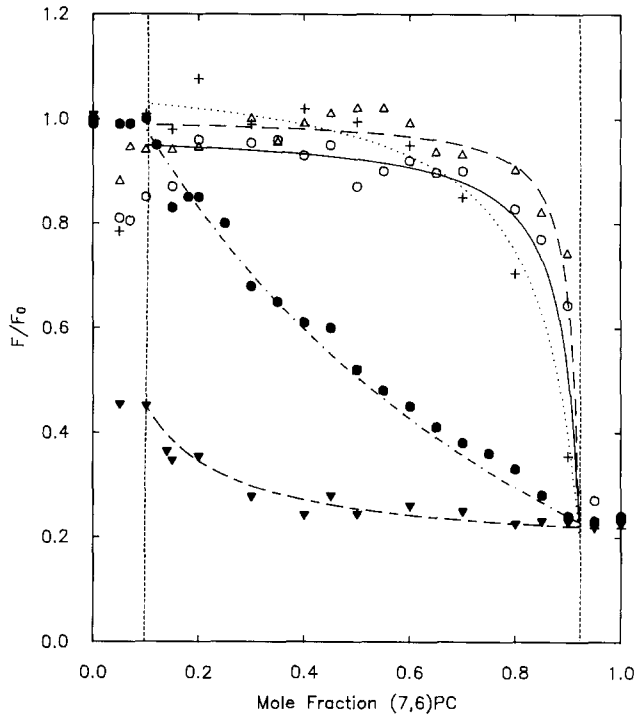

Fig. 2. Fluorescence quenching measurements and calculated quenching curves in DSPC/(7,6)PC mixtures at 21°C for C_nDiI probes for $n=12$ (▼); 16 (●); 18 (+); 20 (△); and 22 (○). The fluorescence measurements, F, are normalized by dividing by F_0, the fluorescence of the probe in the pure gel phase. The curves are calculated in the two-phase region using Eqn. 1 and the range of K_p values that fits the data is shown. Each symbol in the figure marks the average of two measurements. K_p for $n=12$ is 6.0 ± 0.5 (— · —); for $n=16$, 1.8 ± 0.3 (· – · – ·); for $n=18$, 0.09 ± 0.01 (· · · · · ·); for $n=20$, 0.02 ± 0.01 (— — —); and for $n=22$, 0.04 ± 0.01 (———). The calculated partition curves are shown between the phase boundaries which are indicated by the vertical dashed lines.

(7,6)PC with DPPC, DSPC and $Ca(PS)_2$ at 21°C, along with the data for DSPC/(7,6)PC at 35°C. The boundaries of the two-phase gel-fluid regions are indicated in each figure. The $Ca(PS)_2$ case is unique in that the gel phase boundary is at 0 mole fraction (7,6)PC, that is, at the pure $Ca(PS)_2$ phase [18]. All compositions up to 0.56 mole fraction (7,6)PC are in the two-phase region for this case. For the low temperature-induced gel phases (hereafter called thermal gels), DPPC and DSPC, the phase boundaries are taken from the phase diagrams [19]. In these cases there are regions of a single gel phase at low mole fraction (7,6)PC, and a single fluid phase at high mole fraction. Before presenting the results for the partitioning of the C_nDiI probes in the two-phase region, the behavior of the fluorescence of the probes in the single-phase gel region deserves comment.

In Figs. 2, 3 and 5 it is apparent that the fluorescence in some regions of the gel phases is somewhat lower than that at the two-phase boundary. This behavior was unexpected because, although a decreased fluorescence in the presence of increased concentration of spin-

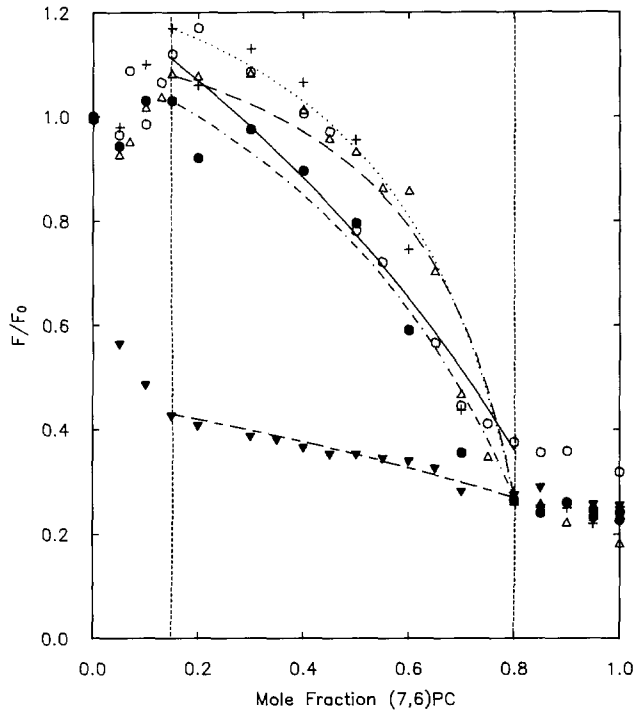

Fig. 3. Fluorescence quenching measurements and calculated quenching curves in DPPC/(7,6)PC mixtures at 21° C for C_nDiI probes for $n = 12$ (▼); 16 (●); 18 (+); 20 (△); 22 (○). The curves are calculated using Eqn. 1. K_p for $n = 12$ is 0.8 ± 0.1 (— · —); for $n = 16$, 0.5 ± 0.1 (· – · – ·); for $n = 18$, 0.35 ± 0.05 (· · · · · ·); for $n = 20$, 0.25 ± 0.05 (— — —); and for $n = 22$, 0.7 ± 0.1 (———).

labelled lipid should occur, it is surprising that the fluorescence actually increases as the composition approaches the two-phase boundary. However, the CnDiI environment in the gel phase is not well-described simply by the overall lipid mole fractions. First, the phase diagrams for DPPC and DSPC mixtures with (7,6)PC both show sharply decreasing solidus lines, a strong indication of nonideality in the gel mixtures [19]. This type of calorimetric behavior can even indicate, in some cases, partial or complete immicibility in the gels at lower temperatures [27,28]. Thus, there is evidence of extensive clustering of (7,6)PC in the gel phase prior to separation into the two-phase fluid-gel region. EPR measurements from 0.02–0.20 mole percent (7,6)PC in gel compared to fluid phase PC support the conclusion of spin label clustering. Fig. 6 shows EPR spectra of 2% (7,6)PC in gel phase DPPC and DSPC. At 2 mole percent (7,6)PC in the gel phase, there is obvious broadening of the EPR spectrum, an indication that there are strong spin–spin interactions. This broadening at such low spin concentrations suggests strongly nonideal mixing of (7,6)PC in these gels.

The behavior of the C_nDiI probes in these nonideal gels is hard to model. The pronounced quenching of the C_{12}DiI probe in the gels (see Figs. 2–5) indicates extensive contact of this short-chain probe with the (7,6)PC. The C_{12}DiI homolog must be clustering in the (7,6)PC domains of the gel. The behavior of the other C_nDiI probes is different from that of the C_{12}DiI: the fluorescence in the gel phase initially decreases, then increases near the two-phase boundary. This trend must be a result of either a decrease in the probability of the probe encountering a (7,6)PC neighbor even as the concentration of spin label increases in the gel, or of a change in the interactions in the bilayer such that the quenching efficiency is lowered. Both cases could lead to the observed increased fluorescence as the spin concentration approaches the two-phase region. We note that even though these observations about the gel state are interesting in themselves, they have no effect on evaluation of the partition coefficients in the two-phase mixtures. The determination of the partition coefficient is dependent simply on the measured fluorescence at the phase boundary [20].

The fluorescence quenching curves in Figs. 2–5 in the two-phase region show the typical monotonic decrease in fluorescence from the gel boundary to the fluid boundary. Eqn. 1 relates the observed fluorescence, F, to the fluorescence of the probe at the gel phase boundary, F_G, and at the fluid phase boundary,

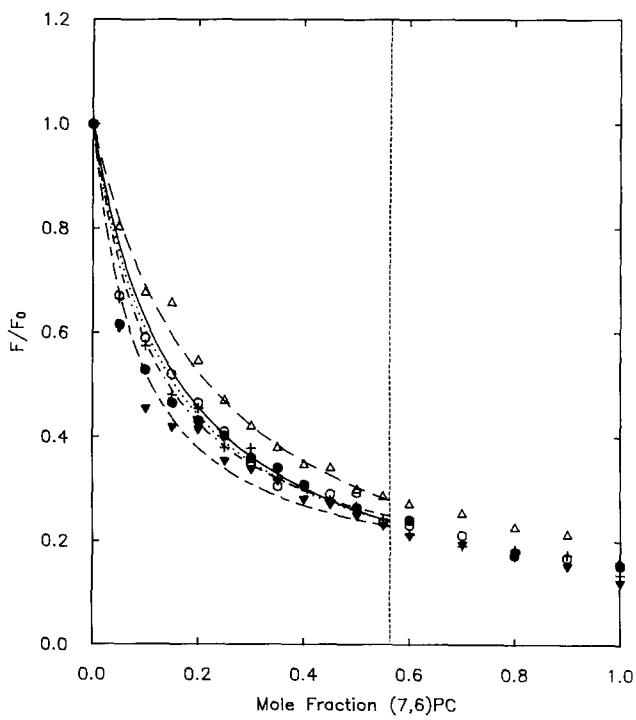

Fig. 4. Fluorescence quenching measurements and calculated quenching curves in $Ca(PS)_2$/(7,6)PC mixtures at 21° C for C_nDiI probes for $n = 12$ (▼); 16 (●); 18 (+); 20 (△); 22 (○). The curves are calculated using Eqn. 1 with K_p for $n = 12$ is 7.5 ± 0.5 (— · —); K_p for $n = 16$, 5.8 ± 0.5 (· – · – ·); for $n = 18$, 5.0 ± 0.8 (· · · · · ·); for $n = 20$, 3.5 ± 0.5 (— — —); and for $n = 22$, 4.5 ± 0.5 (———). The gel phase boundary is at 0 mole fraction (7,6)PC, and the fluid phase boundary indicated by the vertical dashed line.

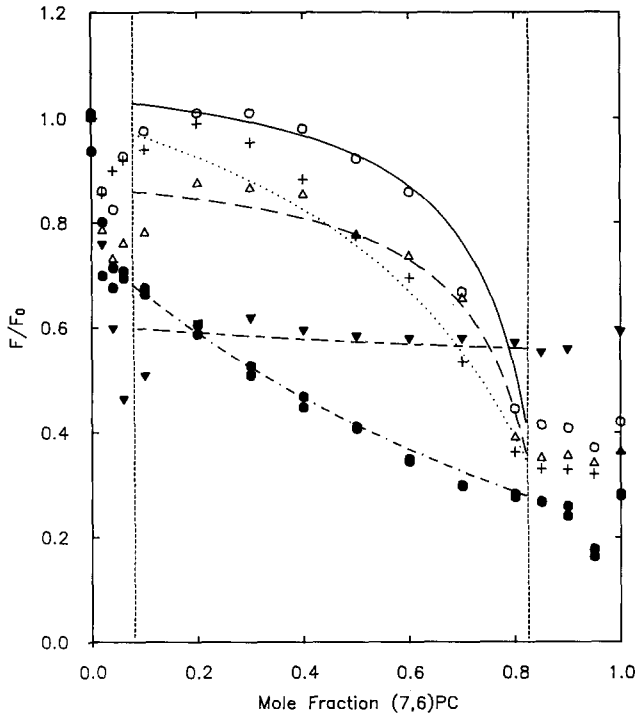

Fig. 5. Fluorescence quenching measurements and calculated quenching curves in DSPC/(7,6)PC mixtures at 35° C for C*n*DiI probes for $n = 12$ (▼); 16 (●); 18 (+); 20 (△); 22 (○). The curves are calculated using Eqn. 1. K_p for $n = 16$, 1.5 ± 0.3 (·–·–·); for $n = 18$, 0.40 ± 0.1 (·····); for $n = 20$, 0.15 ± 0.06 (— — —); and for $n = 22$, 0.20 ± 0.05 (———). K_p for the $n = 12$ probe is not calculated because of the small difference in fluorescence between the gel and fluid boundaries.

F_{LC}, and on the relative amounts and compositions of gel and fluid phases in which the probe is distributed:

$$F = F_{LC} + (F_G - F_{LC})\left[\frac{[G]}{K_p(1-[G])+[G]}\right] \quad (1)$$

where,

$$[G] = \frac{[(7,6)PC]_{LC} - [(7,6)PC]}{[(7,6)PC]_{LC} - [(7,6)PC]_G} \quad (2)$$

Here, $[(7,6)PC]_{LC}$ is the mole fraction at the liquid crystal boundary, $[(7,6)PC]_G$ is the mole fraction at the gel boundary, and [G] is the mole fraction of gel in the mixture with a particular overall mole fraction of quencher, [(7,6)PC]. We have previously determined the location of the phase boundaries of the thermal gel mixtures [19] and of the $Ca(PS)_2$/(7,6)PC system [18]. The partition coefficient is then determined by changing the single variable, K_p, in Eqn. 1 to obtain the best fit of a calculated curve to the experimental quenching curves of F vs. [(7,6)PC] in the two-phase region. (For convenience in plotting, the fluorescence data are normalized by dividing the observed fluorescence, F, by F_0, the fluorescence in the pure gel phase.)

Fig. 2 and 3 show quenching curves for the thermal gels, DPPC and DSPC at 21°C for the $n = 12$ to 22 C_nDiI homologs, and Fig. 5 shows results for DSPC at 35° C. The gel and fluid boundaries are indicated by the vertical dashed lines, and the solid curves through the experimental points are calculated with the K_p indicated in each figure, using Eqn. 1. Reliable values of K_p require a significant difference in probe fluorescence between the phase boundaries. Thus, the K_p for C_{12}DiI at 35° C is practically indeterminate. The estimated uncertainties in K_p are given in the figures.

The quenching curves for the C_nDiI probes in DOPS/(7,6)PC with Ca^{2+} are shown in Fig. 4. The gel and fluid boundaries are 0 and 0.56, respectively [18], so the calculated curves start at 0 and stop at 0.56 mole fraction of spin labelled lipid. In Fig. 7 the quenching of the fluorescence of the five C_nDiI probes in DOPS/(7,6)PC mixtures without calcium ion is shown. Since these lipid mixtures are fluid at all compositions, the quenching curves reflect how the probes behave in the absence of gel phase. The fact that the curves for all probes are virtually superimposable suggests that the lipid composition around each probe in the fluid phase is not strongly dependent upon the alkyl chain length of the probe molecule.

Fig. 8 shows a comparison of the K_p values for the CnDiI probes for the three gel phase systems studied,

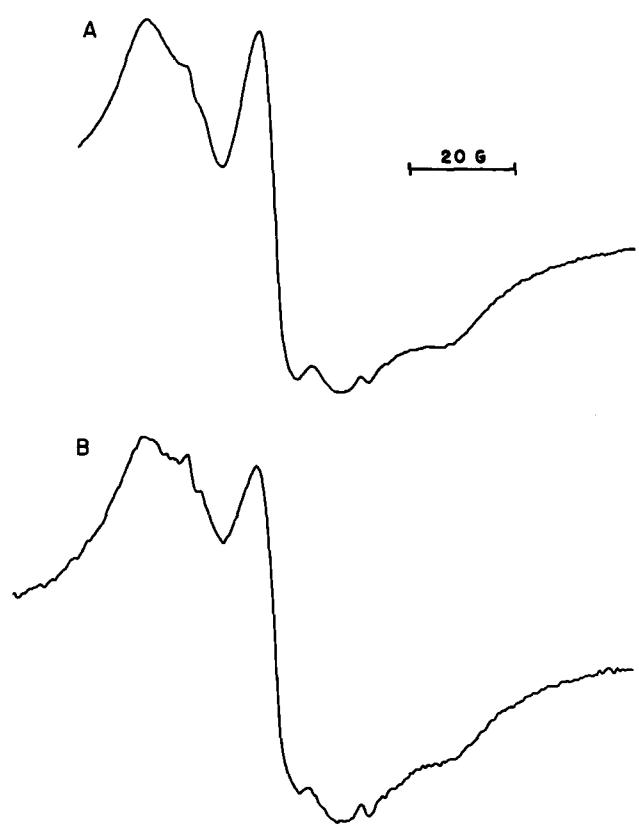

Fig. 6. EPR spectra of two mole percent (7,6)PC in (A) DPPC, and (B) DSPC gel phases at 21° C.

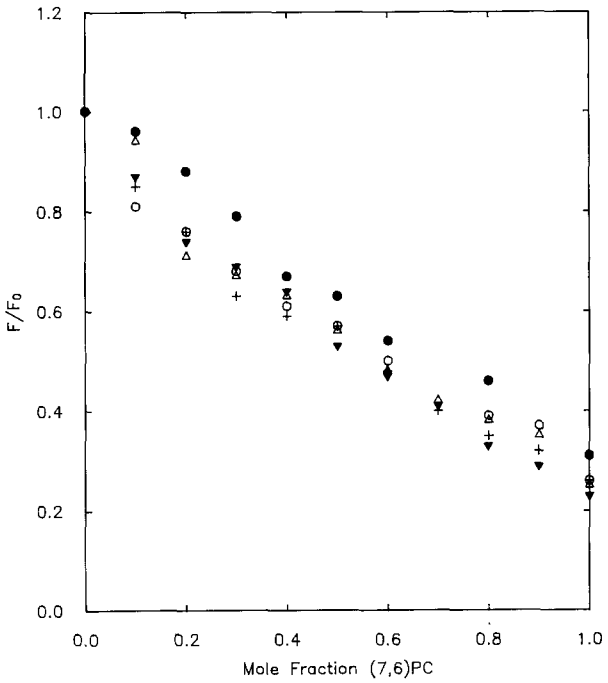

Fig. 7. Fluorescence quenching curves of C_nDiI probes in fluid phase mixtures of DOPS and (7,6)PC. $n = 12$ (▼); 16 (●); 18 (+); 20 (△); 22 (○).

plotted as log K_p vs. n. For all gel phases there is a minimum in K_p observed near $n = 20$, which means that the partitioning into the gel phase is optimal near that value. There are, however, significant differences in the magnitude of K_p for the three gels with the C_{20} probe. For $Ca(PS)_2$, K_p is 3.5, while for the thermal gels K_p is 0.25 and 0.02 for DPPC and DSPC, respectively. On the other hand, at 21°C the $n = 12$ probe in both the $Ca(PS)_2$ and DSPC partitions little into the gel phase, the K_p values being 7.5 and 6.0, respectively. The corresponding K_p for DPPC is 0.8, showing virtually no preference for gel or fluid phase. In the $Ca(PS)_2$ mixtures there is little change in the partition coefficient with n, and the values show favorable partitioning into the fluid phase lipid for every length of probe chain. Also in the DPPC case there is only a small change in K_p with chain length of the probe, but here all probes partition slightly in favor of the gel phase. The DSPC gel phase is interesting because of the relatively large change in K_p from the C_{12} to the C_{20} probe. It is clear that the particular character of the gel state strongly determines probe partition.

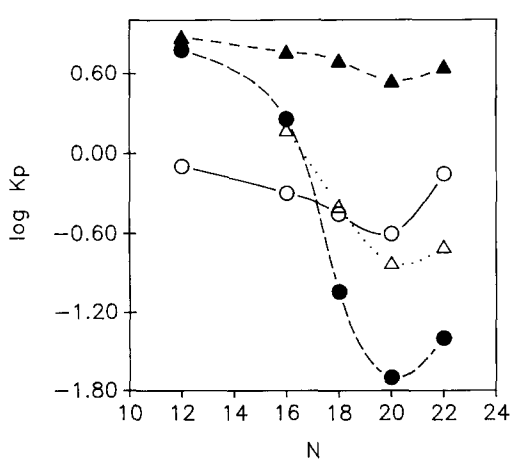

Fig. 8. Plot of log partition coefficient (K_p) vs. N, the number of carbon atoms in indocarbocyanine dye, C_NDiI, in DPPC (○), DSPC (●), and $Ca(PS)_2$ (▲) gel phases mixed with (7,6)PC fluid phase at 21° C, and DSPC (△)/(7,6)PC mixtures at 35° C.

Discussion

In order to understand the partitioning behavior of the C_nDiI probes, several factors should be considered. First, the structure of the probe obviously affects the extent of partitioning into fluid or gel phase. In addition, the characteristics of both the gel and fluid phases themselves will influence the distribution of the probe. Of these factors the probe structural features and the character of the gel phase are probably dominant, because there is evidence that the interaction of the various probes with exclusively fluid lipids is not strongly selective (Fig. 7). In our treatment, we will neglect the difference among the fluid phase mixtures of (7,6)PC with DSPC, DPPC, and DOPS. As justification for this simplified treatment, several partitioning studies have shown that the fluid phase characteristics do not control the value of K_p. For example, Lentz et at. [13] found a K_p value of 1.0 for the distribution of diphenylhexatriene (DPH) between the DPPC gel phase and DMPC fluid phase. This number agrees quite well with the K_p value of 1.5 for DPH partitioning between DPPC gel and (7,6)PC fluid phase found by Florine and Feigenson [18]. Also, we have recently measured K_p for C_{12}DiI in DSPC/POPC mixtures and found a value of 0.15 ± 0.1 (unpublished results), which is similar to the value of 0.09 ± 0.05 found in this study for the DSPC/(7,6)PC system. So, in the following discussion we assume that the major factors influencing the distribution of the probes between fluid and gel phases are the probe structure and the properties of the gel phase lipid.

Comparison of C_nDiI partitioning with that of other probes

A number of studies of the partitioning of probe molecules between gel and fluid phases have been reported [13,14,18,19,20,24,29,30]. If it is assumed that the transfer of probes into various fluid phases is independent of the type of fluid phase, then the general trend is that probes which perturb the lipid organization in the gel phase are rather strongly partitioned into the fluid phase. For example, the n-(9-anthroyloxy) fatty acids, with the fluorescent moiety at differing locations on the fatty acid chain, have partition coefficients in the range

of 4 to 5 in thermal gels such as DPPC/(7,6)PC or DSPC/(7,6)PC, and near 18 for the $Ca(PS)_2$ gel phase [19]. The only exception to this trend is for the 16-AP probe, which has the anthroyloxy group on the end of the fatty acid chain, and has K_p values of 1.5 for the thermal gels and 11 for the $Ca(PS)_2$ phase. These latter values are very close to those found for DPH in the corresponding systems and suggests that, at least for the thermal gels, the region near the alkyl chain terminus of the lipids is less ordered in the gel phase than the regions farther up the chain [19]. Probes which partition most favorably into the gel phases are those that can fit the constraints of the largely *trans*-configurations of the acyl chains in the gel phase. The *trans*-isomer of parinaric acid has a K_p value of 0.3, favoring thermal gels [14], and about 3 in the $Ca(PS)_2$ phase [18]. This probe can fit better into the gel phase than can those probes with bulky groups.

The partition coefficients for the C_nDiI probes indicate that several of these compounds show strong preference for the gel phase of DSPC and less for DPPC. Except for the C_{12}DiI and C_{16}DiI homologs, all show preference ($K_p < 1$) for partitioning into the thermal gel phases of DPPC and DSPC in mixtures with (7,6)PC. For the especially rigid and orderly $Ca(PS)_2$ gel [18,23,30], the C_nDiI probes all have K_p values that are quite small when compared with other probes studied in these mixtures (see Fig. 8).

In light of the structure of the C_nDiI probes, it is reasonable to expect that the alkyl chains penetrate into the lipid bilayer, while the fluorescent carbocyanine moiety is located near the headgroup region of the bilayer [1,31]. This orientation of the probe would explain why there would be less perturbation of the gel phase with this class of probes relative to others thus far reported. As suggested by Packard and Wolf [31], when the chain lengths of the probes closely match those of the lipids in the bilayer, packing is efficient and thus stronger interchain interactions in the gel provide favorable partitioning into that phase. For the shorter and longer-chained C_nDiI probes, matching with the bilayer thickness is not possible and thus perturbations of the lipid packing occur in the vicinity of the probe, and the magnitude of K_p increases relative to the non-perturbing homologs. The rather dramatic differences in K_p for the C_{12}DiI and the C_{20}DiI probes in DSPC/(7,6)PC mixtures at 21°C illustrate this effect clearly, the values being 6.0 and 0.02 for the two cases, respectively. The chain length dependence of partitioning behavior presented in Fig. 8 shows that for a given lipid mixture the matching of probe alkyl chain carbon length with acyl chain carbon length of the lipid is not exact for maximum partitioning into the gel phase. That is, the smallest K_p (largest gel phase preference) occurs with the probe chain length somewhat longer than that of the bilayer lipid chains. This result could mean that the alkyl chains of the dye match with the acyl chains of the lipid, together with part of the lipid glycerol backbone. It is clear, however, that when the probe chain is three or four carbons longer or shorter than the bilayer thickness, there is a significant decrease in the partitioning into the gel phase. It is also apparent that the particular gel phase properties also play an important role in the behavior of the C_nDiI probes.

Comparison of partitioning in the different lipid mixtures

The higher K_p values for the C_nDiI probes in $Ca(PS)_2$/(7,6)PC mixtures relative to the K_p values in thermal gels is probably related to the unique characteristics of the rigid $Ca(PS)_2$. The interaction of Ca^{2+} with phosphoserines is believed to produce a specific complex with a lamellar structure and with the hydrocarbon chains in a very rigid conformation [23,30]. But, in spite of the structure of the $Ca(PS)_2$ gel phase, the C_{20}DiI probe has a K_p of about 3.5, meaning that about 1 of 4 DiI molecules is in the gel phase if equal volumes of gel and fluid phase are present. This ratio represents one of the highest for partition of a fluorescent probe into the $Ca(PS)_2$ gel phase, the K_p values of most other probes being greater than 10 [18,19].

In the case of the thermal gel mixtures another interesting point emerges. In comparing the K_p values for the probes in DSPC with those for DPPC, it is apparent that the two gel phases have different properties with respect to partitioning behavior, particularly for the C_{12}DiI or C_{20}DiI probes. The matching of the alkyl chain lengths in the probe with bilayer thickness leads to more partitioning of the probe into a DSPC gel than into a DPPC gel. An explanation for this behavior is that the DSPC gel at 21°C has a longer and more orderly profile than does DPPC at the same temperature, leading to more favorable enthalpy of packing for a long alkyl chain. In DPPC the gel phase does not accommodate the probes as readily, even for the shorter probe chains that might be well-matched with bilayer thickness. The interactions in the gel in this case would be less favorable, and lead to larger K_p values.

The temperature dependence of the partitioning for the DSPC gel phase also supports the idea that the gel phase properties are very important in probe partitioning. At 35°C partitioning into the gel phase is considerably less, in fact, leading to K_p values quite similar to those for the DPPC gel. The properties of the DSPC gel at 35°C appear to provide less suitable conditions for partitioning of the probe into the gel. If some of the order and rigidity of the gel were diminished at 35°C, then the introduction of probes into the bilayer would lead to weaker interactions between the probe chains and the lipid acyl chains. The 7-fold increase in K_p in going from 21 to 35°C in DSPC for the C_{20}DiI probe reveals a significant change arising from these weakened interactions. Furthermore, the similarities between the

DSPC results at 35°C and the DPPC results at 21°C, both now about 20° C below their main gel-liquid crystal transition temperatures, imply that the gel characteristics are quite similar with respect to probe partitioning.

The partitioning behavior reflected in the thermal gels implies that at each temperature not only do the phase boundaries change, but also the orderliness in the gel phase is altered. Support for these arguments comes from NMR and Raman studies of the gel phase of synthetic PCs. There is evidence from deuterium NMR lineshapes that motion around the long axes of the acyl chains in DPPC gels is not 'frozen out' until −7°C [32]. This study shows that order parameters continue to decrease below the main gel-fluid transition temperature, and that there is disorder in the acyl chains even to as low as −20 to −30°C. Changes in Raman band intensities with temperature also imply *gauche* conformers well below the main gel-liquid crystal phase transition [33,34]. The angle of tilt of the acyl chains can also change with temperature in phospholipid bilayers [35,36]. Thus, it would clearly be an oversimplification to consider the gel as an invariant solid at all temperatures. The relevance of this behavior to the partitioning of molecules between gel and fluid phases is that the temperature plays a key role in defining the gel phase properties with respect to the distribution of the probes between phases. Thus, not only is the particular lipid important, but also the temperature at which the measurements are carried out.

Thermodynamics of transfer of the C_nDiI probes from gel to fluid phase

It is possible to make a crude estimate of the enthalpy of transfer of the C_nDiI probes from gel to fluid phase using the van't Hoff expression for the temperature dependence of the partition coefficient,

$$\ln\left(\frac{K_p^1}{K_p^2}\right) = \frac{\Delta H^0}{R}\left(\frac{T_1 - T_2}{T_1 T_2}\right) \quad (3)$$

Since we have measured partition at only two temperatures, we recognize that the enthalpy value will be only an estimate. Still, the trends that are revealed in the thermodynamic values are consistent with the ideas presented above. Table I presents the data for four of the five probes studied, the C_{12}DiI homolog not included because of the difficulty in obtaining K_p at 35°C (see Fig. 5). The enthalpy of transfer from the gel to fluid phase is near zero for the C_{16}DiI, but becomes quite positive by the C_{18} derivative. As argued above, it is reasonable to presume that the length of the hydrocarbon chains matches well with the bilayer lipid acyl chains and thus such probes are accommodated well in the gel phase. Stronger interactions should prevail that would be absent in the fluid bilayer lipid, and the enthalpy change for the transfer to the fluid should be endothermic. On the other hand, the probes with shorter chains do not fit as well in the DSPC gel and become disruptive of the acyl chains packing, producing weaker interactions between probe and lipid environment. This disruption would cause the enthalpy of transfer to the fluid to become more exothermic. In Table I the C_{20} derivative has the most positive enthalpy of transfer, while the shorter or longer chain lengths show the trends to less endothermic transfer enthalpies. The entropy values are also consistent with these order-disorder arguments. The shorter probes cause disruption, producing disorder in the vicinity of the probe in the gel state, so large that the entropy change for the transfer to fluid is close to zero for the C_{16} probe. For the longer probes there is less disruption, and the entropy increases in going from the gel to fluid state. The data in Table I show that there must be considerable enthalpy-entropy compensation in the transfer process, since the free energy values are quite small relative to the entropy and enthalpy of transfer. The partition coefficient is determined by a balance between the energy differences for the probe in the two phases, and the specific ordering effects in the probe environment in the gel-fluid mixtures.

TABLE I

Thermodynamic data for the transfer of C_nDiI probes from gel to fluid phase in DSPC at 25°C

ΔH^0 was calculated from the van't Hoff expression for K_p at 21 and 35°C in DSPC. ΔG^0 was determined at 25°C by interpolation of K_p values between 21 and 35°C.

n	ΔH^0 (kcal/mol)	ΔG^0 (cal/mol)	ΔS^0 (cal/mol per K)
16	0.0	−245	+1
18	+19.3	+1187	61
20	26.1	1978	81
22	20.8	1637	64

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References

1 Sims, P.J., Waggoner, A.S., Wang, C.-H. and Hoffman, J.F. (1974) Biochemistry 13, 3315–3330.
2 Fahey, P.F., Koppel, D.E., Barak, L.S., Wolf, D.E., Elson, E.L. and Webb, W.W. (1977) Science (Wash. D.C.) 195, 305–306.
3 Fahey, P.F. and Webb, W.W. (1978) Biochemistry 17, 3046–3053.
4 Peters, R. (1981) Cell Biol. Int. Rep. 5, 733–760.

5 Dragson, P.R., Blumenthal, R. and Handler, J.S. (1981) Nature 294, 718–722.
6 Wolf, D.E., Edidin, M. and Handyside, A.H. (1981) Develop. Biol. 85, 195–198.
7 Wolf, D.E., Kinsey, W., Lennarz, W. and Edidin, M. (1981) Develop. Biol. 81, 133–138.
8 Wolf, D.E. (1988) Biochemistry 27, 860–865.
9 Derzko, Z. and Jacobson, K. (1980) Biochemistry 19, 6050–6057.
10 Klausner, R.D. and Wolf, D.E. (1980) Biochemistry 19, 6199–6203.
11 Ethier, M.F., Wolf, D.E. and Melchior, D.L. (1983) Biochemistry 22, 1178–1182.
12 Yguerabide, J. and Foster, M.C. (1979) J. Membr. Biol. 45, 109–123.
13 Lentz, B.E., Barenholtz, Y. and Thompson, T.E. (1976) Biochemistry 15, 4529–4537.
14 Sklar, L.A., Miljanich, G.P. and Dratz, E.A. (1979) Biochemistry 18, 1707–1716.
15 London, E. and Feigenson, G.W. (1981) Biochim. Biophys. Acta 649, 89–97.
16 Welti, R. and Silbert, D.F. (1982) Biochemistry 21, 5685–5689.
17 Feigenson, G.W. (1983) Biochemistry 22, 3106–3112.
18 Florine, K.I. and Feigenson, G.W. (1987) Biochemistry 26, 1757–1768.
19 Huang, N., Florine-Casteel, K.I., Feigenson, G.W. and Spink, C.H. (1988) Biochim. Biophys. Acta 939, 124–130.
20 London, E. and Feigenson, G.W. (1981) Biochemistry 20, 1932–1938.
21 Portis, A., Newton, C., Pangborn, W. and Papahadjopoulos, D. (1979) Biochemistry 18, 780–789.
22 Ohnishi, S. and Tokutomi, S. (1981) Biol. Magn. Reson. 3, 121–153.
23 Hauser, H., and Shipley, G.G. (1984) Biochemistry 23, 34–41.
24 Florine, K.I. and Feigenson, G.W. (1987) Biochemistry 26, 2978–2983.
25 Kingsley, R.B. and Feigenson, G.W. (1979) Chem. Phys. Lipids 24, 135–147.
26 Caffrey, M. and Feigenson, G.W. (1981) Biochemistry 20, 1949–1961.
27 Mabrey, S.D. and Sturtevant, J.M. (1976) Proc. Natl. Acad. Sci. USA 73, 3862–3866.
28 Lee, A.G. (1977) Biochim. Biophys. Acta 472, 285–344.
29 Foster, M.C. and Yguerabide, J. (1979) J. Membr. Biol. 45, 125–146.
30 Feigenson, G.W. (1986) Biochemistry 25, 5819–5825.
31 Packard, B.S. and Wolf, D.E. (1985) Biochemistry 24, 5176–5181.
32 Davis, J.H. (1979) Biophys. J. 27, 339–358.
33 Yellin, N. and Levin, I.W. (1977) Biochim. Biophys. Acta 489, 177–190.
34 Levin, I.W. and Bush, S.F. (1981) Biochim. Biophys. Acta 640, 760–766.
35 Harlos, K. (1978) Biochim. Biophys. Acta 511, 348–355.
36 Janiak, M.J., Small, D.M. and Shipley, G.G. (1979) J. Biol. Chem. 254, 6068–6078.
37 Tenchov, B.G. (1987) Prog. Surf. Sci. 20, 273–340.