Selectivity of Fluorescent Lipid Analogues for Lipid Domains†

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ABSTRACT: We have examined the phase partition preferences of the even chain length (n = 10–22) diacyl-3,3′-indocarbocyanine iodides (C_n diI) incorporated in disaturated lecithin (PC) vesicles. Two parameters were used to determine this phase preference: (i) the direction of shift of the phase transition temperature (T_m) induced by the dyes and (ii) the self-quenching of fluorescence due to aggregation in the gel phase of those dyes which preferentially partition into the fluid. Dyes that lower T_m preferentially partition into the fluid phase; those that raise T_m preferentially partition into the gel. By these criteria in dimyristoyl-PC, C_{10} diI and C_{12} diI preferentially partition into the fluid phase, C_{14} diI and C_{16} diI show no preferential partition, C_{18} diI preferentially partitions into the gel, and C_{20} diI and C_{22} diI preferentially partition into the fluid. In dipalmitoyl-PC, the pattern of preference is identical with that observed in dimyristoyl-PC, only shifted to longer chain length diI's by two carbons. Diffusion measurements by fluorescence photobleaching recovery of these dyes in gel-phase multilayers showed them all to be immobile, $D < 10^{-10}$ cm^2/s, while in fluid-phase multilayers they all had diffusion coefficients of $D \sim 10^{-8}$ cm^2/s independent of chain length. In mixed-phase multilayers, however, each C_n diI showed mobile fractions which reflected its phase-partition preference.

Cell membranes appear to be organized as lipid bilayers into which proteins are inserted or applied (Oseroff et al., 1973). The bilayer is commonly referred to as "fluid" as if it were in fact a fluid and homogeneous phase. However, as pointed out by White (1978), the orientation of lipid molecules in the bilayer necessitates a greater degree of order and cooperativity than in an ideal fluid. Furthermore, studies of model membranes show that certain lipids do not mix together to form a single homogeneous phase but rather segregate into separate phases, fluid and gel (Shimschick & McConnell, 1973; Verkley et al., 1972; Phillips et al., 1972; Jain & White, 1977; Stewart et al., 1979). Until recently, no such phases or domains have been conclusively demonstrated in cell membranes, though this work with model membranes suggests that they ought to form in native membranes as well. Indeed, there are now indications of their presence in native membranes.

Klausner et al. (1980) have suggested the existence of lipid domains in lymphocytes by measuring the fluorescence lifetime heterogeneity of diphenylhexatriene incorporated into these membrane. They have also shown that exogenously added fatty acids fall into two operational classes which may be correlated with structure, the cis unsaturated fatty acids, which preferentially partition into fluid-phase lipid, the trans unsaturated and saturated fatty acids, which preferentially partition into the gel.

These results suggest that other membrane-soluble molecules should have similar preferential phase partition properties and suggested to us a possible explanation of some fluorescence photobleaching recovery (FPR) measurements on the diffusion of the lipid probes *N*-(4-nitrobenzo-2-oxa-1,3-diazolyl)phosphatidylcholine and 3,3′-di(C_nH_{2n+1})indocarbocyanine iodides (C_n diI)[1] in the sea urchin egg plasma membrane (Wolf et al., 1981). They found either an increase or a decrease in diffusion rate on fertilization dependent on probe structure. In the case of the C_n diI's, this results from differences in the dependence of the diffusion coefficient D and the diffusing fraction on acyl chain length before and after fertilization. This, in turn, could reflect the presence of lipid domains in the membrane, a preferential partitioning of the dyes into different domains which is chain length dependent, and a reordering of these domains on fertilization. This interpretation depends upon the demonstration that the C_n diI's are in fact preferentially soluble in the fluid or gel phase.

Here, we demonstrate that the C_n diI's do preferentially partition into different lipid phases as a function of chain length. In mixed-phase vesicles, this preferential partition results in a dependence of diffusion coefficient and mobile fraction upon chain length similar to that observed by Wolf et al. (1980) in sea urchin eggs. Furthermore, we demonstrate a number of indicators of phase partition preference including the preferential aggregation of diI in unfavorable phases and shifts in the endothermic phase transition of an artificial disaturated lecithin bilayer caused by the dye. Thus, it appears the the C_n diI's may be used not only to probe lateral diffusion of lipids but also to investigate the organization of lipids into domains. Such investigation ought to better define the state of bilayer lipids in cell membranes.

Materials and Methods

Dyes and Lipids. All dyes were synthesized and generously provided by Dr. A. Waggoner (Sims et al., 1974). The structure of the C_n diI's is shown in Figure 1. All of the dyes have identical excitation and emission spectra and quantum yields.

All lipids were obtained from Avanti Biochemicals Inc. (Birmingham, AL). They were >99% pure on thin-layer chromatography. Dyes containing vesicles were made by mixing appropriate molar ratios of dye to lipid in benzene and drying these under argon. Vesicles were made by using a vortex mixer to disperse the dried lipid into a buffer containing 139 mM NaCl, 6 mM KCl, and 10 mM Hepes, pH 7.2 (100 μg/mL), at a temperature 5–10 °C above the T_m of the lipid. The suspension was agitated for 60 s and kept at 4 °C.

Large liposomes for FPR measurements were prepared from 10 to 20 mg/mL solutions of phospholipid in chloroform/methanol (2:1) or ethanol which contained 5×10^{-4} mol fraction of C_n diI/lipid. From this solution, 50–100 μL was dried under nitrogen on an acid-cleaned microscope slide;

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[1] Abbreviations used: C_n diI, 3,3′-di(C_nH_{2n+1})indocarbocyanine iodide; DLPC, dilaurylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; FPR, fluorescence photobleaching recovery.

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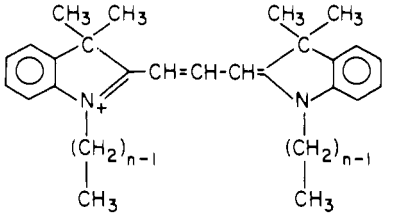

FIGURE 1: Structure of the 3,3′-diacylindocarbocyanine iodides.

100–200 μL of deionized water (10 MΩ cm) prepared with a Milli-Q system, (Millipore, Bedford, MA) was placed over the dried lipid, and the layer was maintained above the longest lipids melting temperature for 5 min. The liposomes were then taken up into 100-μm path length microslides (Vitro Dynamics, Rockaway, NJ) which were sealed with paraffin wax for FPR measurement.

Spectroscopy. Fluorescence of the dye was measured with an Aminco-Bowman spectrofluorometer with 2-nm slits. Excitation was 514 nm, and emission intensity was read at 560 nm. For phase transition studies, the cuvette holder was maintained at 5 °C above the phase transition of the lipid being examined, by the use of circulated water from a Haake temperature-controlled water bath. Microcuvettes containing 150 μL of vesicle suspension (Precision Instruments) were kept on ice in their adaptors. A temperature scan was obtained by monitoring the fluorescence after placing the iced cuvette and adaptor into the cuvette holder. Temperature was monitored by a digital microprobe placed into the cuvette but kept above the light path. The temperature and the fluorescence intensity were then simultaneously recorded on an x-t strip chart recorder.

Absorbance spectra were recorded with a Carey Model 15 scanning spectrophotometer.

Analysis. Transition temperature information was recorded as follows. The fluorescence intensity of the C_n diI dyes showed sharply defined changes at the endothermal phase transition of the disaturated lipid into which they were incorporated (Figure 1). Three discrete slopes could be drawn along the intensity vs. temperature curve, one giving the temperature dependence of the intensity in the gel phase, another showing the intensity through the transition, and a third representing the intensity as a function of temperature in the fluid phase. The intersections of the first and second and the second and third curves gave the temperatures of the beginning (T_1) and end (T_2) of the phase transition. The temperature width of the phase transition $\Delta T = T_2 - T_1$. T_m was defined as the temperature that corresponded to the fluorescence intensity halfway between the fluorescence at T_1 and T_2.

The formalism using colligative solution theory that relates the direction of shift of the lipid-phase transition to the phase partition of a molecule dissolved in the lipid was given in Klausner et al. (1980). Briefly, if γ equals the ratio of the mole fraction of solvent found in the gel phase to the mole fraction found in the fluid phase, then

$$T_m = T_0\left(\frac{\Delta H^\circ}{\Delta H^\circ + RT_0 \ln \gamma}\right)$$

T_m is the measured phase transition temperature, T_0 is the phase transition without solute, and ΔH° is the enthalpy of the phase transition. Therefore, if a solute preferentially partitions in the gel phase, the mole fraction of solvent will be higher in the fluid phase, γ will be less than 1, and T_m will be greater than T_0.

Fluorescence Photobleaching Recovery (FPR) Measurements. In FPR, the fluorescence from a small spot on a uniformly fluorescent membrane is monitored by a focused laser beam of low intensity. Light intensity is briefly increased 10^3–10^4-fold which causes significant (typically 50%–70%) bleaching of the fluorescence. The light is then returned to monitoring levels, and the recovery of fluorescence due to diffusion of unbleached molecules into the spot is observed. Two parameters are obtained from FPR measurements, the fraction of dye molecules free to diffuse and the half-time for recovery of fluorescence from which one obtains the diffusion coefficient, D (Axelrod et al., 1976). Our apparatus is similar to the one described by Koppel et al. (1976), except that we monitor continuously. We used a Control 2.5-W argon–krypton laser, a Leitz Ortholux II fluorescence microscope, an EMI 978A photomultiplier tube, and a PAR 1140A quantum photometer. Data were recorded on a Hewlett–Packard 320 dual channel dc amplifier recorder. For our experiments, we used the 531-nm laser line focused to an exp(−2) radius of (1.1 + 0.2 μm). Monitoring intensities were ~3 μω, and bleaching times were 5–20 m s. The temperature was regulated by using a Bailey TS-2 temperature controller and stage.

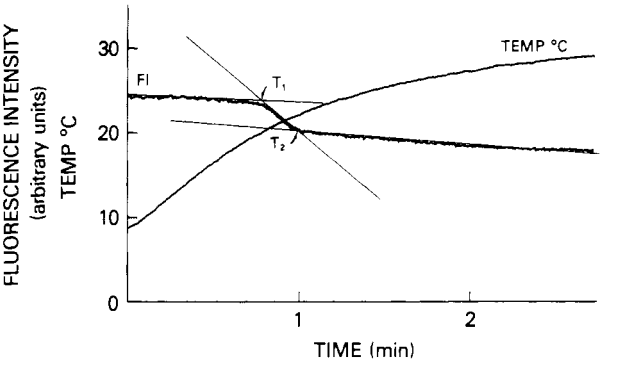

FIGURE 2: Fluorescence intensity (FI) vs. temperature (TEMP °C) for 0.1 mol fraction of C_{14} diI in DMPC (100 μg/mL) vesicles. Three discrete slopes can be drawn along the FI vs. temperature curve, one giving the temperature dependence in the gel phase, another during the transition, and another in the fluid phase. These curves intersect as shown at T_1 and T_2. We define $\Delta T = T_2 - T_1$ as a measure of the width of the transition. The transition temperature T_m is then given by the temperature at which the FI is half way between FI at T_1 and T_2.

Results

Preferential Phase Partition of the C_n diI's in Dimyristoylphosphatidylcholine (DMPC). In order to determine the preference of the different C_n diI's for the gel vs. fluid phase of DMPC, we measured the effect of adding a 0.1 mol fraction of the dye on the melting temperature of the lipids, T_m (for pure DMPC, T_m = 23 °C; Pagano & Weinstein, 1978). We found that fluorescence intensity can be used to monitor the endothermic phase transition of the lipid. As we shall discuss further below, the fluorescence intensity of the diI's in lipid vesicles is strongly dependent on temperature and exhibits a discontinuity centered at the melting temperature T_m. At low dye concentrations, this discontinuity is centered at a T_m equal to that of the pure lipid. Figure 2 shows the fluorescence intensity of DMPC vesicles containing 0.1 mol fraction of C_{14} diI as a function of temperature. We observed a marked decrease in fluorescence intensity beginning at T_1 = 21.0 °C, centered at T_m = 22.0 °C, and ending at T_2 = 22.8 °C. Since C_{14} diI does not alter T_m, we conclude that C_{14} diI has no significant preference between the two phases of DMPC.

Similar intensity vs. temperature profiles for DMPC vesicles containing 0.1 mol fraction of each of the C_n diI's for even n's from 10 to 18 are shown in Figure 3, and the results are

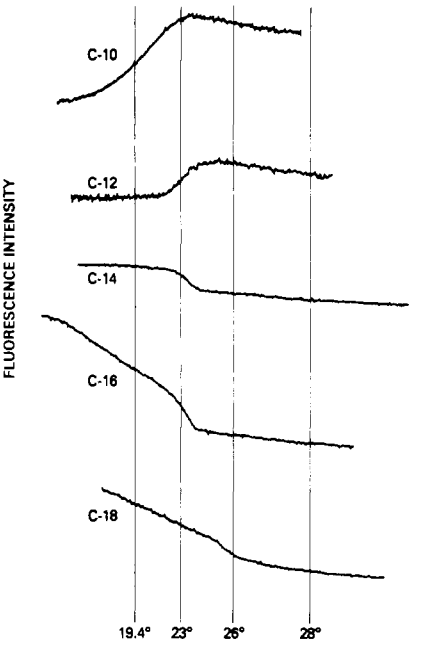

FIGURE 3: Fluorescence intensity vs. temperature profiles for 0.1 mol fractions of the C_n diI's in DMPC (100 μg/mL) vesicles (n = 10–18).

Table I: Phase Transition Data for DMPC Vesicles Containing a 0.1 Mol Fraction of C_n diI

probe	T_1 (°C)	T_2 (°C)	T_m (°C)	ΔT (°C)	FI(10 °C)/FI-(27 °C)
C_{10} diI	13.3	23.5	19.4	10.2	0.68
C_{12} diI	22.0	24.6	23.3	2.6	0.89
C_{14} diI	21.0	22.8	22.0	1.8	1.29
C_{16} diI	22.5	24.8	23.4	2.3	1.89
C_{18} diI	25.3	26.2	25.8	0.9	1.59
C_{20} diI	18.0	22.7	20.8	4.7	0.88
C_{22} diI	18.0	22.7	20.9	4.7	0.79

tabulated in Table I. On the basis of transition temperature alone, only the $T_m(C_{10})$ = 19.4 °C and $T_m(C_{18})$ = 25.8 °C differ significantly from the T_m = 23 °C of the pure lipid. We thus conclude that C_{10} diI prefers the fluid phase of DMPC while C_{18} diI prefers the gel. Notice also that the broad $\Delta T = T_2 - T_1$ = 10.2 °C of C_{10} diI and the narrow ΔT = 0.9 °C for C_{18} diI are consistent with their preference for gel or fluid (see Klausner et al., 1980). C_{12} diI, C_{14} diI, and C_{16} diI all show no preference as judged by the alteration in T_m.

The C_n diI's may be further divided on the basis of the direction of the effect of temperature on fluorescence intensity (FI). At a 0.1 mol fraction, C_{10} diI and C_{12} diI fluorescence intensities increase on going through the phase transition while C_{14} diI, C_{16} diI, and C_{18} diI show decreases. This effect is concentration dependent. At 0.1 mol fraction, FI(10 °C)/FI(27 °C) = 0.68 for C_{10} diI and 1.59 for C_{18} diI. At 0.002 mol fraction, FI(10 °C)/FI(27 °C) = 2.0 for both C_{10} diI and C_{16} diI. A mole fraction of 0.002 does not significantly perturb the DMPC T_m for any of the dyes tested. A possible explanation for the different intensities would be if there were different partitions for each dye between the aqueous phase (where their quantum yields are extremely low) and the gel or fluid lipid phases. Since all the dyes have essentially identical quantum yields in organic solvents, it is reasonable to assume that their partition in the lipid should be reflected in their fluorescence intensity in the vesicles. However, when we compare the ratio of the fluorescence intensities of 0.002 mol fraction of C_{10} diI or C_{16} diI in DMPC, either below or above the phase transition, they are identical. Therefore, this

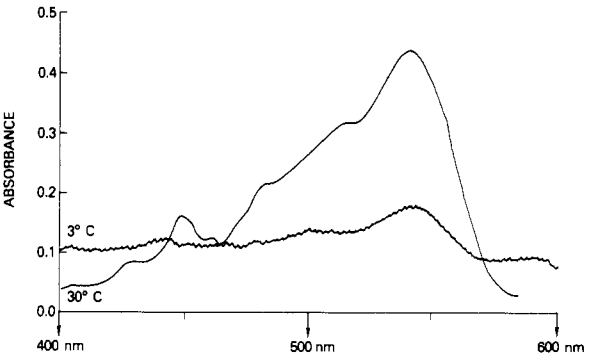

FIGURE 4: Absorbance spectra of a 0.1 mol fraction of C_{10} diI in DMPC (100 μg/mL) vesicles at 3 °C (below the phase transition) and at 30 °C (above it). The reference cuvette contained an identical sample of DMPC vesicles only without C_{10} diI.

concentration-dependent reversal of the effect of the lipid phase on fluorescence intensities of C_{10} diI and C_{12} diI does not reflect an altered aqueous to lipid partition coefficient compared to the longer chain diI dyes.

A second possibility is that dyes like C_{10} diI which markedly prefer the fluid phase of DMPC aggregate at high concentrations in the gel phase which results in self-quenching. To test this hypothesis, we prepared DMPC vesicles with 0.1 mol fraction of C_{10} diI, cooled them to 3 °C, measured the absorbance spectrum, raised the temperature through T_m to 30 °C, and measured the absorbance spectrum again. These spectra are shown in Figure 4. They are remarkably similar to the spectra of Sims et al. (1974) for the related dye 3,3'-dipropylthiocarbocyanine as a function of concentration. At 30 °C we observed the ~540-nm peak due to monomers of C_{10} diI and a lesser peak at ~500 nm due to dimers. At 3 °C the overall absorbance spectrum was diminished, and a third peak (the so-called J band; Sheppard, 1942) appeared at ~590 nm due to the formation of larger aggregates. As demonstrated by Sims et al. (1974), these aggregates result in significant fluorescence quenching. Thus, C_{10} diI, which prefers the fluid phase of DMPC, aggregates and is quenched when forced to be in the gel. A similar quenching is observed for C_{12} diI. Thus, on the basis of this criterion, C_{12} diI also prefers the fluid phase.

In summary, in DMPC the C_n diI's tested with acyl chain lengths less than that of the lipid (n = 14) preferentially partition into the fluid while those with $n \geq 14$ either partition preferentially into the gel (as we showed unambiguously for C_{18} diI) or show no preference.

Preferential Partition of the C_n diI's in Dipalmitoylphosphatidylcholine (DPPC). On the basis of our observation in DMPC, we predicted that in DPPC vesicles the behavior of the C_n diI's would be the same but that the pattern would be shifted by two carbons. The data for C_n diI's for even n = 10–18 in DPPC at 0.1 and 0.01 mol fraction are given in Table II. As predicted at 0.1 mol fraction, C_{10}–C_{14} diI show a preference for fluid with T_m's less than that of the pure lipid (41 °C, Pagano & Weinstein, 1978) while C_{16} diI and C_{18} diI show T_m's at about 41 °C. Furthermore, the narrowing of ΔT with increasing n is indicative of a shift from preference for fluid to preference for gel. Also, as predicted, at 0.1 mol fraction we observed an increase in fluorescence intensity on going through the phase transition for $n < 16$ and a decrease for $n > 16$. [Compare FI(30 °C)/FI(40 °C) in Table II for a 0.1 mol fraction.] As in the case of DMPC, at low concentrations all of the dyes show identical decreases in fluorescence intensity on going through the transition temperature (compare the ratios FI(30 °C)/FI(40 °C) in Table

Table II: Phase Transition Data for DPPC Vesicles Containing C_n diI

probe	T_1 (°C)	T_2 (°C)	T_m (°C)	ΔT (°C)	FI (40 °C)/FI (30 °C)
		0.1 Mol Fraction of C_n diI			
C_{10} diI	36.0	41.0	39.1	5.0	2.05
C_{12} diI	33.8	41.0	39.1	5.2	1.93
C_{14} diI	38.4	40.5	39.7	2.1	1.02
C_{16} diI			40.7[a]		0.66
C_{18} diI			41.1		0.84
		0.01 Mol Fraction of C_n diI			
C_{10} diI			41.6		0.77
C_{12} diI	no data				
C_{14} diI			39.8		0.72
C_{16} diI			40.6		0.78
C_{18} diI			41.0		0.74

[a] Where no data are given, only one sharp break was observed. In such cases, the temperature where this break occurred was taken as T_m.

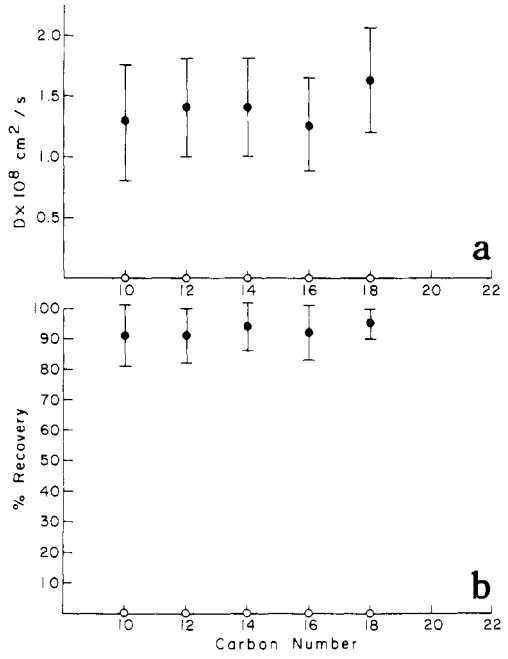

FIGURE 5: Diffusion coefficients (a) and percent recoveries (b) as a function of acyl chain length at 10 °C in DLPC vesicles (closed circles) and DPPC (open circles) vesicles.

II for a 0.01 mol fraction of the different C_n diI's). Thus, dyes with acyl chain length less than that of the lipid ($n = 16$) prefer the fluid and aggregate and self-quench when forced to be in the gel while those with n greater than that of the lipid either show no preference or prefer the gel.

Lateral Diffusion of C_n diI's in Single Phase Liposomes. The C_n diI's were incorporated at 5×10^{-4} mol fraction into large dilaurylphosphatidylcholine (DLPC) liposomes and large DPPC liposomes; the diffusion coefficient and fraction diffusing were measured by FPR at 10 °C (DLPC is a fluid at 10 °C and DPPC a gel). The results are shown in Figures 5 and 6a,b,d,e, and in DLPC all of the C_n diI's showed essentially complete recovery with $D \approx 1.2 \times 10^{-8}$ cm²/s. In contrast, in DPPC all of the C_n diI's showed essentially no recovery (fraction recovery ≤ 20%; $D \leq 5 \times 10^{-11}$ cm²/s).

The Diffusion of C_n diI's in Two Phase Liposomes. C_n diI's were incorporated at 5×10^{-4} mol fraction into large 1:1 DLPC/DPPC liposomes. These liposomes are known to be mixed phase at 10 °C with domains of about 300-nm diameter. The diffusion coefficients and fraction diffusing are shown in

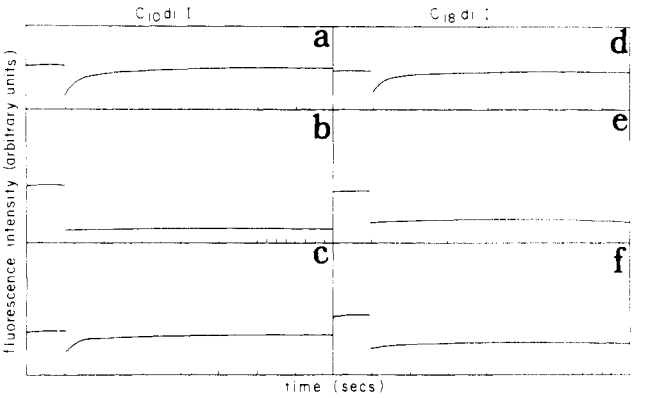

FIGURE 6: Diffusion of C_{10} diI and C_{18} diI in single- and mixed-phase vesicles at 10 °C. (a) C_{10} diI in DLPC, $D = 1.1 \times 10^{-8}$ cm²/s and % recovery = 100; (b) C_{10} diI in DPPC, $D < 10^{-10}$ cm²/s, % recovery = 0; (c) C_{10} diI in a 1:1 mixture of DLPC/DPPC, $D = 1.0 \times 10^{-8}$ cm²/s, % recovery = 100; (d) C_{18} diI in DLPC, $D = 1.6 \times 10^{-8}$ cm²/s, % recovery = 100; (e) C_{18} diI in DPPC, $D < 10^{-10}$ cm²/s, % recovery = 0; (f) C_{18} diI in 1:1 mixture of DLPC/DPPC, $D = 0.8 \times 10^{-8}$ cm²/s, % recovery = 12.

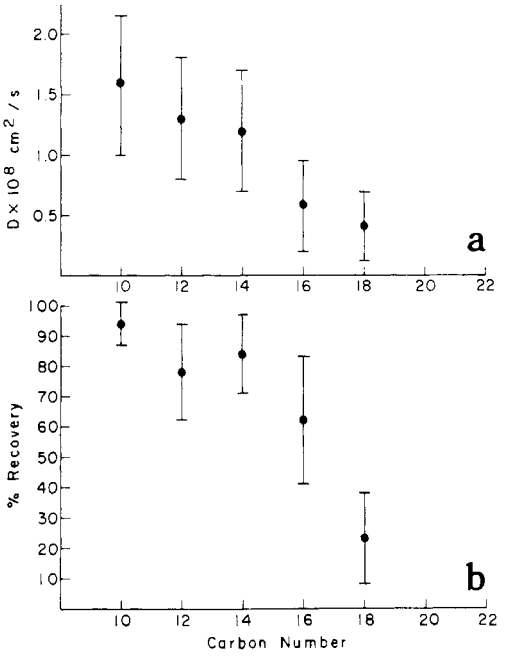

FIGURE 7: Diffusion coefficients (a) and percent recoveries (b) of the C_n diI's as a function of acyl chain length at 10 °C in mixed-phase vesicles (a 1:1 mixture of DLPC/DPPC) at 10 °C.

Figure 6c,f and Figure 7. C_{10} diI, which we know prefers the fluid, diffused just as it did in DLPC. C_{18} diI, which we know prefers the gel, diffused with $D \sim 4 \times 10^{-9}$ and only ~25% free to diffuse. That is, it diffused almost as slowly as if it were in the gel. Intermediate chain lengths showed intermediate behavior, indicating a transition from fluid to gel preference.

The Preferential Partition of Longer Chain Length C_n diI's. One would not predict this shift from fluid to gel preference to be monotonic for longer chain length. It is hard to imagine for instance that C_n diI's with $n > 28$ would take on an all-trans configuration in DMPC, thus projecting through the bilayer into the aqueous phase on the opposite side. Rather, it would prefer to fold up into the bilayer, which would be more easily accomplished in the fluid than in the gel phase. Thus, we predicted that at some $n > 18$ reversion to fluid preference would be observed. As shown in Figure 8 and Table I, this is true for both C_{20} diI and C_{22} diI. By all criteria, T_m, ΔT, and gel quenching of fluorescence in 0.1 mol fraction DMPC

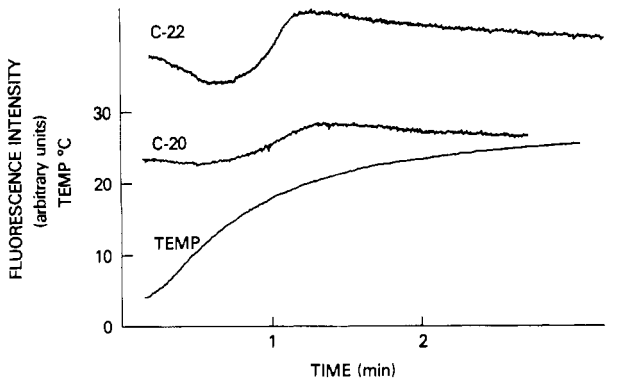

FIGURE 8: Fluorescence intensity of C_{20} diI and C_{22} diI as a function of temperature in DMPC (100 μg/mL).

liposomes, these dyes exhibit preferential partition into fluid-phase lipid.

Discussion

In this paper we have shown that the C_n diI's show a chain-length-dependent phase preference in lipid bilayers. C_n diI's with acyl chains shorter than the lipid prefer the fluid and aggregate when forced to be in the gel. C_n diI's with acyl chains comparable to the lipid exhibit no preference. C_n diI's with acyl chains somewhat longer than the lipid prefer the gel phase while those much longer prefer the fluid.

This dependence can be understood in terms of a simple model. When the chain lengths are comparable, the diI and lipid will be indistinguishable to the first order and the diI should pack equally well into either phase. As diI chain length increases, it will pack best into the extended all-trans gel bilayer. As noted above, this cannot go on indefinitely. In the extreme where n is greater than the bilayer thickness, the diI will have to fold up, and this is most easily accomplished in the fluid phase. As we have observed, this return to a fluid preference occurs before this extreme. For n's much shorter than the lipid's, one would expect head group interactions to dominate and for the diI's to prefer the more disordered state where they will not interfere with crystalline packing. One would, as we have in fact observed, predict that in order for the acyl chains to align into the close-packed arrays of the gel state they will exclude the short-chain-length diI's into domains or aggregates themselves.

This nonmonotonic dependence of phase preference of the C_n diI's in lipid bilayers points to an important conclusion about the incorporation of molecules into the bilayers, that is, that phase partition is dependent not only on hydrophobicity but also on structure. This has now been demonstrated here for the C_n diI's, by Klausner et al. (1980) for the free fatty acids and by Pringle & Miller (1979) for long-chain alcohols. A lipid bilayer cannot be treated as a bulk phase. As pointed out by White (1978), it is a highly ordered and cooperative structure, and miscibility will depend on the ability of a molecule to fit into that order. This must apply not only to exogenously added molecules but to the natural constituents of the membrane themselves. Thus, it is not unreasonable to expect lipid domains in natural membranes.

As noted in the introduction, there is some evidence for the existence of lipid domains in natural membranes (Klausner et al., 1980; Brown et al., 1977). We have shown that the existence of lipid domains is sufficient to explain some of the chain-length dependence of C_n diI diffusion in sea urchin eggs (Wolf et al., 1980). Klausner et al. (1980) have shown that domains exist in lymphocyte plasma membrane. Dragsten (personal communication) has failed to see differences in C_n diI (n = 10–22) diffusion in lymphocytes. However, as we have shown, gel preference is exhibited for a narrow range of n. The sea urchin eggs used by Wolf et al. (1980) live at 15 °C while lymphocytes live at 37 °C. While in sea urchin eggs extrema in the chain length dependence for the diI's might reasonably occur at n = 12 or 14, one would expect them to occur at $n > 20$ in lymphocytes. It may be that longer or different probes are needed to detect domains in lymphocytes and mammalian cells in general.

The ability to detect domains by FPR will depend on domain size. The 300-cm interdigitated domains (Hui & Parsons, 1975) of the DLPC/DPPC system are probably optimal for the geometry of the laser beam in our FPR machine. Small islands of gel diffusing in a fluid would probably be indistinguishable from monomers by FPR. Similarly, small lakes of fluid in a gel would be indistinguishable from a total gel.

We have demonstrated that lipid domains in membranes can be detected by selective partitioning of fluorescent lipid analogues into different domains and measuring the restrictions which the domains impose on the diffusion of these analogues in the membrane. These studies emphasize that the partition preference of a probe molecule must be known before the probe can be used to meaningfully report upon the structure of membranes.

Acknowledgments

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