Partition of Parinaroyl Phospholipids in Mixed Head Group Systems†

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ABSTRACT: Partitioning of 1-palmitoyl-2-*cis*-parinaroylphosphatidylcholine, 1-palmitoyl-2-*cis*-parinaroylphosphatidylethanolamine, 1-oleoyl-2-*trans*-parinaroylphosphatidylcholine, and 1-oleoyl-2-*trans*-parinaroylphosphatidylethanolamine between solid-phase phosphatidylcholine and fluid-phase phosphatidylethanolamine and between solid-phase phosphatidylcholine and fluid-phase phosphatidylethanolamine is investigated. The partitioning of these probes is shown to be nearly independent of their head group. Fluorescence polarization of the above probes plus *trans*-parinaric acid, *cis*-parinaric acid, 1-palmitoyl-2-*trans*-parinaroylphosphatidylcholine, 1-oleoyl-2-*cis*-parinaroylphosphatidylcholine, and 1-oleoyl-2-*cis*-parinoylphosphatidylethanolamine is examined in phospholipids isolated from LM cell (mouse fibroblast) plasma membranes. A fraction of these phospholipids undergoes a gel–liquid-crystalline phase transition beginning near the physiological temperature. The parinaroyl probes with the highest solid–fluid-phase partition coefficients [Welti, R., & Silbert, D. F. (1982) *Biochemistry* (preceding paper in this issue)] detect the transition most readily. The degree of response to this transition also appears to depend on the acyl chains of the parinaroyl phospholipid rather than the probe head group. This is further demonstrated with the phosphatidylcholine and phosphatidylethanolamine fractions from LM cell plasma membranes.

In the preceding paper (Welti & Silbert, 1982) the determination of the partition properties of eight parinaroyl phospholipids between solid- and fluid-phase phosphatidylcholines was described. In this paper these studies are extended to determine if the nature of the phospholipid head group making up the solid and fluid phases influences the partition properties of the probes. This question is relevant to understanding the relative effects of head group and acyl chains on the tendency of the components of phospholipid mixtures in model systems or biological membranes to "group together" and form phase-separated domains.

The partition of two PC[1] and two PE probes is investigated in two highly immiscible synthetic phospholipid systems: a high-melting PC with a low-melting PE and a high-melting PE with a low-melting PC. The probes 1-16:0,2-cPnPC, 1-16:0,2-cPnPE, 1-18:1,2-tPnPC, and 1-18:1,2-tPnPE were chosen because they partitioned nearly equally between solid and fluid phases in the PC system (preceding paper), and thus their partition coefficients could be determined with the least error.

In a second set of experiments, fluorescence polarization of the parinaroyl phospholipids is examined in phospholipids isolated from the plasma membrane of mouse LM cells. These membranes include a variety of phospholipid head group classes, but PC, PE, and sphingomyelin make up 86% of the phospholipid (Rintoul et al., 1979). A portion of these membrane phospholipids undergoes a gel to liquid-crystalline transition beginning just above the physiological temperature (37 °C) (Welti et al., 1981). The extent to which probes with varying acyl chains and head groups detect this transition is examined in these partially miscible biological phospholipids.

Materials and Methods

Parinaroyl Phospholipids. These were synthesized and purified as described in the preceding paper (Welti & Silbert, 1982).

Model Phospholipids. Dipalmitoylphosphatidylethanolamine and dilinoleoylphosphatidylethanolamine were synthesized and purified by the methods outlined in the preceding paper. The synthesis and purification of DPPC and PDPC are also described there.

Dispersions. All dispersions were prepared by the ethanol-injection method (Kremer et al., 1977). Final phospholipid concentration was 41.5 nmol/mL, and the phospholipid to probe ratio was about 100:1. For the DPPC/DLiPE system, the phospholipids and probe were injected in 15 μL of ethanol into 3.2 mL of 10 mM Tris buffer, pH 7.5, containing 0.31 mg/L BHT at 50 °C. DPPE/PDPC mixtures containing 1 μg of BHT were injected in 20 μL of ethanol into 4 mL of 115 mM NaCl/10 mM Hepes buffer, pH 7.5, at 70 °C. Because the DPPE was somewhat insoluble in ethanol, the ratios of DPPE to PDPC in the mixtures were verified by extracting the dispersions after fluorescence measurements, separating DPPE from PDPC by TLC in chloroform/methanol/water (65:25:4), scraping the spots charred at 150 °C after application of 50% sulfuric acid, and assaying this silica gel for phosphate (Ames, 1966). Plasma membrane phospholipids with the probes and 1 μg of BHT were injected in 15 μL of ethanol into 3.2 mL of 115 mM NaCl/10 mM Hepes buffer, pH 7.5, at 40 °C. In all cases blanks were prepared in the same way as the samples except the probe was deleted.

Absorption and Fluorescence Spectroscopy. Methods are essentially the same as described in the preceding paper. Scattering was very low in all samples except some of those with DPPE in which the OD was about 0.2. No correction was made for scattering in calculation of polarization ratios. Some of the polarization curves in this paper show irreproducible undulations to which no significance is attached. The loss of parinaric absorbance during measurements in the

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[1] Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; cPn, *cis*-parinaroyl; tPn, *trans*-parinaroyl; BHT, butylated hydroxytoluene; EDTA, ethylenediaminetetraacetic acid; DPPC, dipalmitoylphosphatidylcholine; PDPC, 1-palmitoyl-2-docosahexaenoylphosphatidylcholine; DPPE, dipalmitoylphosphatidylethanolamine; DLiPE, dilinoleoylphosphatidylethanolamine; Tris, tris(hydroxymethyl)aminomethane; Hepes, *N*-(2-hydroxymethyl)piperazine-*N*′-2-ethanesulfonic acid; TLC, thin-layer chromatography.

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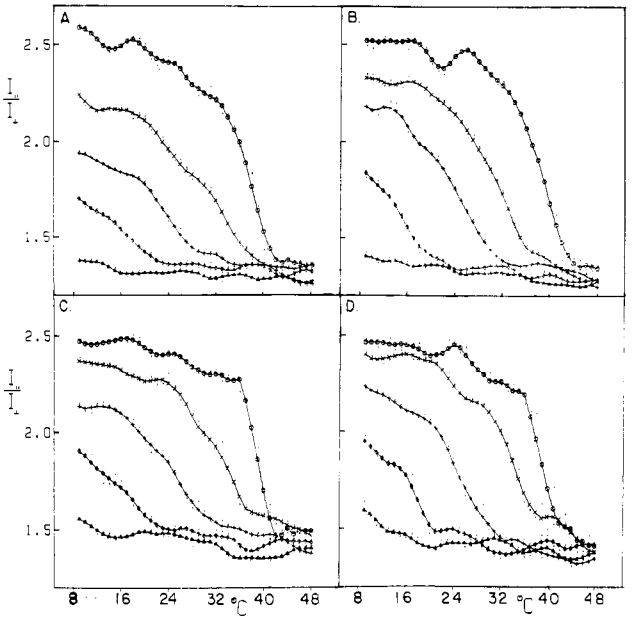

FIGURE 1: Fluorescence polarization in DPPC/DLiPE mixtures as a function of temperature. The probes are (A) 1-16:0,2-cPnPC, (B) 1-16:0,2-cPnPE, (C) 1-18:1,2-tPnPC, and (D) 1-18:1,2-tPnPE. The symbols represent smoothed data, while the actual data points (used in the partition coefficient calculations) are represented by small dots: (△) 0% DPPC; (◇) 25% DPPC; (+) 50% DPPC; (×) 75% DPPC; (○) 100% DPPC. Vesicles were made by ethanol injection; the buffer is 10 mM Tris, pH 7.5.

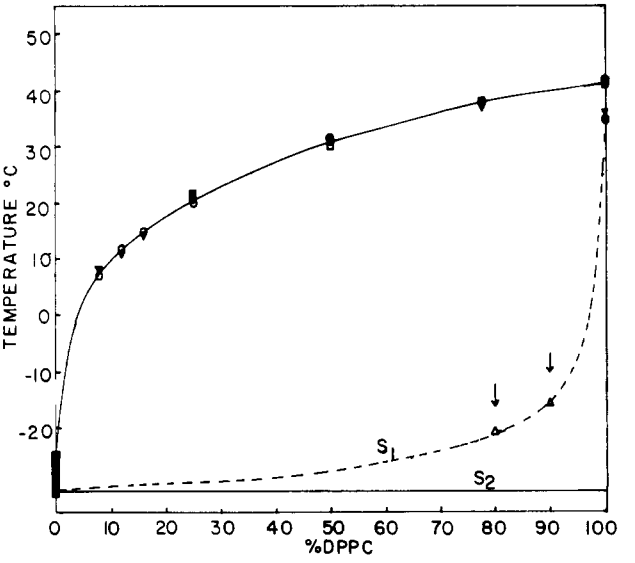

FIGURE 2: DPPC/DLiPE phase diagram. The fluidus line represents the onset of solid-phase formation as detected by the indicated probes. The solidus line represents the completion of solid-phase formation. Solidus line 1 (S_1) represents the upper limit for this line, while solidus line 2 (S_2) represents the case of complete solid-phase immiscibility. Partition coefficients are calculated by using S2. (▼) 1-18:1,2-tPnPC; (○) 1-18:1,2-tPnPE; (□) 1-16:0,2-cPnPC; (●) 1-16:0,2-cPnPE; (△) 1-18:1,2-cPnPC.

DPPC/DLiPE system and the LM cell plasma membrane phospholipids ranged from 0 to about 40% and was typically about 25%. Losses in the DPPE/PDPC system were higher, and thus partition coefficients were not calculated from these data.

Partition Coefficients. These were calculated from the DPPC/DLiPE system by the method described under Results. Values of X_s and X_f at each temperature, for each DPPC/DLiPE mixture, were derived from the phase diagram constructed as described under Results.

LM Cells and Plasma Membrane Preparation. LM cells (mouse fibroblasts), obtained from ATCC (CCL 1.2), were maintained in suspension culture in modified Higuchi's medium as described by Saito et al. (1977). Plasma membranes were isolated after nitrogen cavitation for cell lysis as described previously (Welti et al., 1981).

Isolation of Phospholipids from LM Cell Plasma Membranes. All the plasma membranes used for the experiments presented in this paper came from a single plasma membrane preparation. Lipids were extracted, separated into neutral (sterol-containing) and polar (phospholipid-containing) fractions, and analyzed as described by Rintoul et al. (1979) with the exception that the total phospholipid phosphate was measured by the procedure of Ames (1966).

Fractionation of LM Plasma Membrane Phospholipids. Phospholipids were separated into pure phosphatidylcholine and phosphatidylethanolamine by thin-layer chromatography on 250-μm silica gel G plates. Three solvent systems were used sequentially: after each solvent system the spots were scraped, eluted, and respotted in the next system. The systems were, first, chloroform/methanol/58% ammonium hydroxide (65:25:5), second, chloroform/acetone/methanol/glacial acetic acid/water (30:40:10:10:5), and, third, chloroform/methanol/water (65:25:4).

Results

Fluorescence Polarization of Probes in DPPC/DLiPE Mixtures and DPPC/DLiPE Phase Diagram. Fluorescence polarization of 1-16:0,2-cPnPC, 1-16:0,2-cPnPE, 1-18:1,2-tPnPC, and 1-18:1,2-tPnPE in mixtures of DPPC and DLiPE are shown in Figure 1. A phase diagram for this binary mixture constructed by the method of Sklar et al. (1979) is shown in Figure 2. The points on the fluidus curve represent the onset of solid-phase formation as detected by an increase in the polarization ratio. Polarization data for mixtures with low amounts of DPPC are not shown.

The solidus curve should represent the temperature below which the mixture is completely solid. With DLiPE in 50% glycerol/50% Tris buffer, no transition onset was detected at or above −25 °C. Again using the glycerol solution, we also attempted to determine the temperature at which mixtures reach their limiting solid-phase polarization value. 1-18:1,2-cPnPC was used as a probe since it is especially sensitive to small amounts of fluid phase. Significant amounts of fluid-phase lipid remain in an 80% DPPC mixture at −21 °C and in a 90% DPPC mixture at −16 °C. Thus the true solidus curve lies below the one designated as S_1 in Figure 2. S_2 represents complete solid-phase immiscibility and is likely to be a reasonable approximation of the true solidus line (Sklar et al., 1979). S_2 is used in the partition coefficient calculations that follow.

Calculation of Partition Coefficients in the DPPC/DLiPE System. Partition coefficients calculated according to eq 3 and 4 of the preceding paper (Welti & Silbert, 1982) are shown in Table I. Again, each datum used in the calculations is the quantum yield or polarization ratio of a particular DPPC/DLiPE mixture at a particular temperature. The amount of solid phase in each mixture at each temperature was calculated from the phase diagram by the lever rule (Lee, 1977). The partition coefficients determined at temperatures at which the composition was changing rapidly were excluded from the determination of the mean values.

In general, the polarization values obtained and the partition coefficients calculated in this high-melting PC/low-melting PE system are similar to those determined in the binary PC system (preceding paper). No preferential partitioning of the

Table I: Apparent Partition Coefficients in the DPPC/DLiPE System[a]

	mean $K_p^{s/f}$ (eq 3)	mean $K_p^{s/f}$ (eq 4)	median $K_p^{s/f}$ (eq 3)	median $K_p^{s/f}$ (eq 4)
18:1tPnPC	0.35 ± 0.10 (60)	0.92 ± 0.17 (60)	0.34 (74)	0.91 (74)
18:1tPnPE	0.58 ± 0.60 (60)	1.24 ± 0.48 (60)	0.40 (74)	1.16 (74)
16:0cPnPC	0.60 ± 0.22 (60)	1.24 ± 0.37 (60)	0.56 (74)	1.26 (74)
16:0cPnPE	0.79 ± 0.32 (60)	1.62 ± 0.34 (60)	0.69 (74)	1.68 (74)

[a] The numbers after the ± are 1 standard deviation. The numbers in parentheses are the number of data used in the calculation.

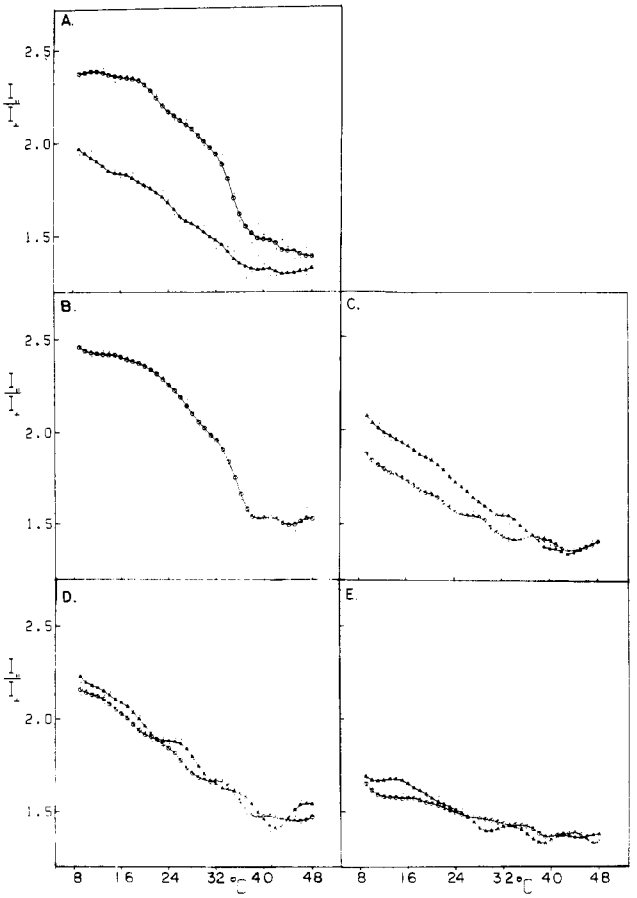

FIGURE 3: Fluorescence polarization of free parinaric acids and parinaroyl phospholipids in LM cell plasma membrane phospholipids. Vesicles were made by ethanol injection; the buffer is 115 mM NaCl/10 mM Hepes, pH 7.5. The probes are as follows: (A) (○) tPnA, (△) cPnA; (B) (○) 1-16:0,2-tPnPC; (C) (○) 1-16:0,2-cPnPC, (△) 1-16:0,2-cPnPE; (D) (○) 1-18:1,2-tPnPC, (△) 1-18:1,2-tPnPE; (E) (○) 1-18:1,2-cPnPC, (△) 1-18:1,2-cPnPE.

parinaroyl phospholipids into phases with the same head group is seen. In fact, just as in the DPPC/PDPC system, the PE probes partition into the solid PC to a slightly greater extent than the PC probes.

Fluorescence Polarization and Partitioning of Probes in DPPE/PDPC Mixtures. In the converse experiment, using high-melting PE and low-melting PC model lipids, we measured the polarization of 1-16:0,2-cPnPC, 1-16:0,2-cPnPE, 1-18:1,2-tPnPC, and 1-18:1,2-tPnPE in mixtures of DPPE/PDPC (data not shown). Again, the partition coefficients of the two PC and two PE probes were similar. Partition coefficients, however, were not quantitatively determined for the DPPE/PDPC system because of idiosyncrasies in the quantum yield data in samples containing DPPE.

LM Cell Plasma Membrane Phospholipids. Figure 3 shows the fluorescence polarization of the two parinaric acids and seven of the parinaroyl phospholipids in phospholipids isolated from mouse fibroblast (LM cell) plasma membranes. These phospholipids undergo a phase separation below about 40 °C, when a fraction of the phospholipids solidify. As shown in the figure, the probes detect this transition to different extents. The probes with the highest partition coefficients in the PC model system (preceding paper) detect the transition most readily, while the probes with low partition coefficients barely detect the transition. As seen from the plots of P vs. X_s (Figure 2 of the preceding paper), this implies that we are observing the onset of gel-phase formation in largely fluid lipid. Again in this mixed head group system, the parinaroyl phospholipids behave as one might expect on the basis of their DPPC/PDPC system partition coefficients (preceding paper). There is little evidence that the head groups of the probes affect their partitioning.

Isolated PC and PE from LM Cell Plasma Membranes. In Figure 4A,B, the fluorescence polarization of 1-18:1,2-tPnPC and 1-18:1,2-tPnPE in phosphatidylcholine and phosphatidylethanolamine isolated from LM cell plasma membranes is shown. Neither probe detects a phase transition in isolated PC, while both probes detect gel-phase formation below about 24 °C in isolated PE. In part C of Figure 4 fluorescence polarization of the probes in an equimolar mixture of isolated PC and PE is shown. Although the gel phase formed in this mixture is likely to be enriched in PE, the PC

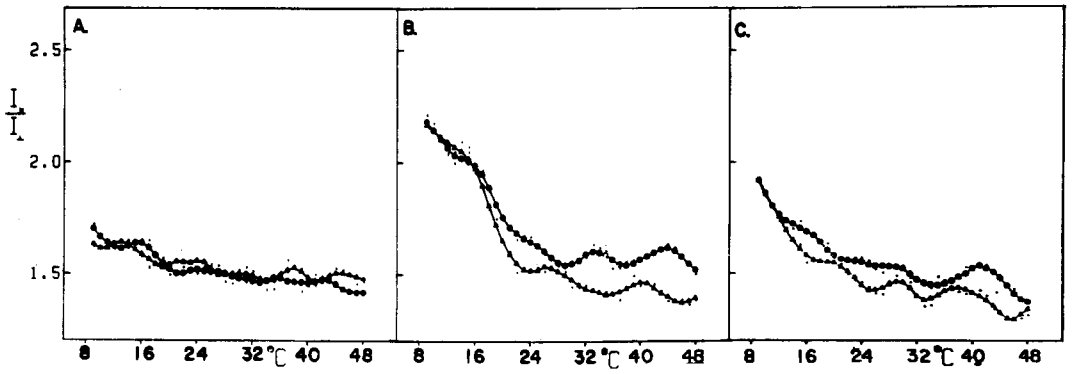

FIGURE 4: Fluorescence polarization of 1-18:1,2-tPnPC and 1-18:1,2-tPnPE in LM cell plasma membrane PC and PE as a function of temperature. Vesicles were made by ethanol injection; the buffer is 115 mM NaCl/10 mM Hepes, pH 7.5. In each part of the figure (○) indicates polarization of 1-18:1,2-tPnPC, while (△) represents polarization of 1-18:1,2-tPnPE. The lipids are (A) LM cell plasma membrane PC, (B) LM cell plasma membrane PE, and (C) A 1:1 (mol/mol) mixture of LM cell plasma membrane PC and PE.

and PE probes detect it to the same extent.

Discussion

Parinaroyl phospholipids provide useful models for biological phospholipids. They provide a reasonable method for studying the factors important in phospholipid domain formation, by allowing the examination of their partitioning among phospholipids of different head group classes. One can determine whether interaction of phospholipids with others of the same head group is an important factor in domain formation.

The experiments in this paper demonstrate the importance of acyl chains on the partitioning behavior of phospholipids. This is shown first in the binary PC and PE mixtures. In these mixtures neither the PC nor PE probes show preference for a phase with like head group. In fact, the PE compared to the PC probes continue to show the same relative preference for the higher melting phase that they showed in the binary PC model system, even when the solid phase is PC and the fluid phase is PE.

The generality of these results is not clear. One could invoke the idea that, with the somewhat disruptive acyl chains of the 18:1,tPn and 16:0,cPn phospholipids, acyl chains are "restrictive" for partitioning into gel-phase lipids. Perhaps when the acyl chains are more like those of the solid-phase lipid, the head group structure becomes important. For example, 16:0,tPn acyl chains resemble di-16:0 acyl chains more strongly than those used in the studies here. One could imagine that without the "restriction" of bulky acyl chains to prevent incorporation into a DPPE phase the head group could become important. Thus 1-16:0,2-tPnPE may "fit" substantially better into gel phase DPPE than 1-16:0,2-tPnPC. This hypothesis remains to be tested.

As mentioned under Results, problems with determination of the quantum yields and, in particular, the loss of probe absorbance during the measurements in sodium chloride/Hepes buffer prevented us from calculating the actual partition coefficients in the DPPE/PDPC system. PE was in general more difficult to work with than PC. Light scattering was greater in PE samples than in PC samples, and in DPPE samples, the quantum yield varied significantly with salt concentration. PE is known to undergo a lamellar-hexagonal phase transition and, in the case of saturated phosphatidylethanolamines, high sodium chloride concentrations have been shown to decrease the temperature of this transition (Harlos & Eibl, 1981). This aspect of PE behavior may also be manifested in the partition coefficients calculated for the DPPC/DLiPE system. The partition coefficients calculated with the total quantum yields of the DPPC/DLiPE mixtures [eq 4, preceding paper (Welti & Silbert, 1982)] are higher than those calculated with the polarization values of the mixtures (eq 3, preceding paper). One explanation for this phenomenon is that the fluid phase present in the DPPC/DLiPE mixtures has different fluorescent parameters than pure DLiPE. This seems reasonable since pure DLiPE probably exists in a hexagonal phase while DLiPE saturated with DPPC may exist in a bilayer liquid-crystalline phase (Cullis & DeKruijff, 1978, 1979). A higher quantum yield for this DPPC-saturated DLiPE phase would increase the quantum yield of the mixtures, increasing the partition coefficients calculated with the total quantum yields. Preliminary experiments indicate that the quantum yields of both pure PDPC and fluid-phase DPPC/DLiPE mixtures are approximately 20% higher than that of pure DLiPE. The polarization ratios are not detectably different. Recalculation of the partition coefficient data with a higher quantum yield value for the fluid phase present in the DPPC/DLiPE mixtures gives better agreement between the partition coefficients calculated by the two equations. It should be stressed, however, that the absolute value of the partition coefficients does not affect the conclusion that the PC and PE probes partition similarly relative to each other in this mixed head group model system compared to their behavior in the PC/PC model system (preceding paper).

Data on the parinaroyl phospholipid probes in LM cell plasma membrane phospholipids generally confirm that acyl chains are the important factor in phospholipid partitioning in this partially miscible mixed head group system. The utility of having probes with a wide range of partition coefficients is demonstrated in that, for example, while 1-16:0,2-tPnPC can readily detect the onset of solid-phase formation, the 1-18:1,2-cPnPC data demonstrate that there is much fluid phase remaining throughout the temperature range investigated. Use of these probes in tandem allows one to define the phase behavior of a biological system more completely than can be done with probes that report only the average properties of the heterogeneous lipid mixture in a membrane.

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