Partition of Parinaroyl Phospholipid Probes between Solid and Fluid Phosphatidylcholine Phases†

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ABSTRACT: The partitioning of parinaroyl phospholipid probes between solid- and fluid-phase phospholipid is examined. The immiscible model system dipalmitoylphosphatidylcholine and palmitoyldocosahexaenoylphosphatidylcholine is used. Fluorescence quantum yields and fluorescence polarization are used to calculate $K_p^{s/f}$, the solid to fluid phase partition coefficient of each probe [Sklar, L. A., Miljanich, G. P., & Dratz, E. A. (1979) *Biochemistry 18*, 1707–1716]. Like free *trans*-parinaric acid (9,11,13,15-*all-trans*-octadecatetraenoic acid), 1-palmitoyl-2-*trans*-parinaroylphosphatidylcholine and 1-palmitoyl-2-*trans*-parinaroylphosphatidylethanolamine partition strongly into solid phase with mean $K_p^{s/f}$s of 7 ± 4 and 9 ± 5, respectively. Like free *cis*-parinaric acid (9,11,13,15-*cis,trans,trans,cis*-octadecatetraenoic acid), another group of phospholipid probes prefer fluid phase just slightly or partition equally between fluid and solid phases. The $K_p^{s/f}$ of 1-palmitoyl-2-*cis*-parinaroylphosphatidylcholine is 0.6 ± 0.2, that of 1-palmitoyl-2-*cis*-parinaroylphosphatidylethanolamine is 1.0 ± 0.3, that of 1-oleoyl-2-*trans*-parinaroylphosphatidylcholine is 0.6 ± 0.3, and that of 1-oleoyl-2-*trans*-parinaroylphosphatidylethanolamine is 0.7 ± 0.3. Two probes partition more strongly into fluid-phase phospholipid. These are 1-oleoyl-2-*cis*-parinaroylphosphatidylcholine with a $K_p^{s/f}$ of 0.2 ± 0.1 and 1-oleoyl-2-*cis*-parinaroylphosphatidylethanolamine with a $K_p^{s/f}$ of 0.4 ± 0.4.

cis- and *trans*-parinaric acids (9,11,13,15-*cis,trans,trans,cis*- and *all-trans*-octadecatetraenoic acids) are fluorescent fatty acids useful as probes of membrane lipid structure [for example, Sklar et al. (1975), Tecoma et al. (1977), and Rintoul et al. (1978)]. When added to an aqueous dispersion of phospholipids, these parinaric acid isomers partition between the aqueous and lipid phases but are only fluorescent in the hydrophobic lipid environment. Fatty acid probes intercalate between the acyl chains of lamellar-phase phospholipids with their carboxyl groups near the aqueous phase (Hubbell & McConnell, 1969; Sklar, 1976). Fluorescence of parinaric acid is sensitive to the physical state of the phospholipids with both quantum yield and polarization increasing sharply as the phospholipids undergo a transition from fluid to solid phase (Sklar et al., 1977a).

The distribution of cPnA[1] and tPnA between fluid and solid lipid phases has been investigated (Sklar et al., 1979). These authors chose the binary system DPPC and PDPC, lipids that exhibit considerable solid-phase immiscibility, to investigate this property. They defined the partition coefficient $K_p^{s/f}$, describing the distribution of PnA between the coexisting solid and fluid phases, as

$$K_p^{s/f} = (X_s^p/X_s)/(X_s^p/X_f) \quad (1)$$

where X_s and X_f are the mole fractions of solid- and fluid-phase lipid present and X_s^p and X_f^p are the mole fractions of the probe in the solid and fluid phases. $K_p^{s/f}$ for tPnA was determined to be 4 ± 1, and $K_p^{s/f}$ for cPnA was determined to be 0.7 ± 0.2. Thus, when fluid and solid phases coexist, tPnA partitions strongly into the solid phase while cPnA slightly prefers the fluid phase.

In this paper the partition properties of eight phospholipid derivatives of *cis*- and *trans*-parinaric acids are similarly characterized. These probes have the advantage of partitioning negligibly into the aqueous phase in aqueous/lipid systems under conditions generally used in membrane studies. It is demonstrated that, like tPnA, 1-16:0,2-tPnPC and 1-16:0,2-tPnPE partition strongly into solid-phase lipid when solid and fluid phases coexist. Like cPnA, 1-18:1,2-tPnPC, 1-18:1,2-tPnPE, 1-16:0,2-cPnPC, and 1-16:0,2-cPnPE distribute equally between the phases or prefer the fluid phase slightly. On the other hand, 1-18:1,2-cPnPC and 1-18:1,2-cPnPE partition more strongly into fluid-phase phospholipid.

Materials and Methods

Fluorescent Fatty Acids. cPnA was the generous gift of Dr. Robert Simoni. tPnA was obtained from Molecular Probes, Inc., Plano, TX, by Dr. C. M. Jackson.

Phospholipid Preparation. Both DPPC and PDPC, as well as the 2-parinaroylphosphatidylcholines and 2-parinaroylphosphatidylethanolamines, were prepared by the following methods. Care was used throughout to avoid exposure of the docosahexaenoyl and parinaroyl derivatives to light or oxygen. L-α-Glycerophosphocholine–cadmium chloride adduct was prepared from Sigma commercial-grade egg PC (Chadha, 1970). Fatty acid anhydrides were prepared by the method of Selinger & Lapidot (1966) from fatty acids obtained from Sigma or Nu Chek Prep. Acylation of L-α-glycerophosphocholine–$CdCl_2$ adduct and of lysophosphatidylcholine by the fatty acid anhydrides was performed by the method of Gupta et al. (1977). All reagents used in the acylation reaction were anhydrous; the glycerophosphocholine–$CdCl_2$ adduct and the lysophosphatidylcholines were dried over phosphorus pentoxide under vacuum at 100 °C overnight. Lysophosphatidylcholine

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[1] Abbreviations: cPnA, *cis*-parinaric acid; cPn, *cis*-parinaroyl; tPnA, *trans*-parinaric acid; tPn, *trans*-parinaroyl; PC, phosphatidylcholine; PE, phosphatidylethanolamine; DPPC, dipalmitoylphosphatidylcholine; PDPC, 1-palmitoyl-2-docosahexaenoylphosphatidylcholine; $I_\parallel$ and $I_\perp$, the intensities measured parallel and perpendicular to the exciting light; P, the polarization ratio ($P = I_\parallel/I_\perp$ with the values corrected for instrumental anisotropy and background fluorescence, as determined by blanks containing no probe); Tris, tris(hydroxymethyl)aminomethane; TLC, thin-layer chromatography; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid.

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was prepared by the action of *Crotalus adamanteus* venom (phospholipase A_2) (Sigma) on PC in a stirring two-phase system of one part 2% MeOH in ether and one part 20 mM $CaCl_2$/10 mM Tris-HCl buffer, pH 7.4. Phospholipase D, prepared as described by Christie (1973), was used to convert PC to PE (Comfurius & Zwaal, 1977). Phospholipids were purified by dry-column elution chromatography or preparative TLC on silica gel G, both using as a developing solvent chloroform/methanol/water (65:25:4) with 0.5 mg/L butylated hydroxytoluene. The dry-column chromatography technique involves elution of lipids loaded onto the top of a bed of tightly packed dry silica gel. This technique gives good resolution, and the separations are analogous to TLC with the same solvent system. Absorption spectra of the phospholipid probes in ethanol were virtually identical with those of the parinaric acid isomer from which they were derived (Sklar et al., 1977b).

Phospholipid Dispersions. Dispersions were prepared by the ethanol-injection method (Kremer et al., 1977). Ten microliters of ethanol containing 166 nmol of DPPC and/or PDPC, approximately 0.1 nmol of butylated hydroxytoluene, and 1 nmol of probe was injected into 4 mL of 115 mM NaCl/10 mM Hepes buffer, pH 7.5 at 55 °C. Blanks were prepared without probe. Two 0.4-mL aliquots were removed from each sample for phosphate assay (Ames, 1966), and the remaining 3.2 mL was used for fluorescence and absorbance measurements. Dispersions were bubbled with nitrogen, and light was excluded from the samples.

Absorption Spectroscopy. Spectra were recorded before and after fluorescence measurements on a Cary 118 UV–vis scanning spectrophotometer. Blank lipid samples prepared as described above had an OD of less than 0.10 at 320 nm; thus the scattering was negligible in terms of causing depolarization (Lentz et al., 1979). Absorbances used in calculation of quantum yields were averages of the absorbances before and after fluorescence temperature scans. From 0 to 50% of the absorbance was lost during a temperature scan. This loss of absorbance and how it was handled are discussed further under Discussion.

Fluorescence Spectroscopy. Quantum yield and fluorescence measurements were performed on a Spex Fluorolog spectrofluorometer. The excitation wavelength was 320 nm for probes derived from tPnA and 325 nm for probes derived from cPnA in phospholipid dispersions. The excitation slit width was 5 nm due to the fixed entrance slit used, but the monochromator slit band-pass was set at 2.5 nm to reduce the intensity of the exciting beam. Emission was measured at 420 nm with a 40-nm band-pass. Temperature scans were performed by cooling the cuvette at a rate of 1 °C/min as the sample was stirred magnetically. Only a slight amount of hysteresis is observed at this rate of temperature change when heating and cooling curves are compared (Sklar, 1976). A Neslab Endocal RTE 5 bath, a Neslab ETP-3 temperature programmer, and a Markson digital thermometer with a thermocouple were used to regulate the cooling. The sample compartment was continually flushed with nitrogen blowing over the sides of the cuvette to eliminate water condensation. Excitation was in the vertical plane. Vertical and horizontal emission intensities were collected manually from the digital output of the fluorometer. P values were calculated, smoothed, and plotted by computer. The correction for instrumental anisotropy described by Azumi & McGlynn (1962) and used by Sklar et al. (1979) was used in calculating P values. The method of Savitzky & Golay (1964) was employed, a nine-point quadratic cubic least-squares fit for smoothing the data being used. In the polarization plots shown in this paper the large symbols are smoothed data while the small symbols are unsmoothed data. Unsmoothed data were used in all partition coefficient calculations. The polarizer alignment was verified with fluorescein in alkaline glycerol (Chen & Bowman, 1965), which gave a value of $P = 2.51 \pm 0.04$. Thus there was a larger amount of depolarization within our apparatus than within that of Sklar et al. (1979), who obtained a value of 2.81 ± 0.03.

Fluorescence quantum yields and partial quantum yields of the phospholipid probes were calculated as

$$Q_{\parallel},\ Q_{\perp},\ \text{or}\ Q_{tot} = \frac{I_{\parallel},\ I_{\perp},\ \text{or}\ I_{tot}}{\text{absorbance of sample at exciting wavelength}} \times \frac{A(st)}{I_{tot}(st) \times 0.02} \quad (2)$$

where $A(st)$ and $I_{tot}(st)$ refer to the absorbance and intensity of a stock solution of cPnA in ethanol at 25 °C with excitation at 320 nm. $I_{tot} = I_{\parallel} + 2I_{\perp}$, and thus $Q_{tot} = Q_{\parallel} + 2Q_{\perp}$ (Sklar et al., 1979). In the calculation of the quantum yields of the free fatty acid probes, the absorbance of the sample at the exciting wavelength was corrected by subtracting the fraction of the absorbance due to probe in the aqueous phase. The lipid–water partition coefficients and extinction coefficient determined by Sklar et al. (1979) were used.

Partition Coefficients. Solid–fluid phase lipid partition coefficients were determined by measuring the total quantum yields or polarization ratio, P, of each probe in mixtures of DPPC and PDPC. $K_p^{s/f}$ can be calculated at temperatures below the onset of solid-phase formation.

The solid–fluid partition coefficient of each probe is calculated according to two equations, one employing total quantum yields and the other employing partial quantum yields. The equations, developed by Sklar et al. (1979), are

$$K_p^{s/f} = (P^{mix}Q_{\perp}^{f} - Q_{\parallel}^{f})X_f/[(Q_{\parallel}^{s} - P^{mix}Q_{\perp}^{s})X_s] \quad (3)$$

and

$$K_p^{s/f} = (Q_{tot}^{mix} - Q_{tot}^{f})X_f/[(Q_{tot}^{s} - Q_{tot}^{mix})X_s] \quad (4)$$

where $Q_{\parallel}^{f}$, $Q_{\perp}^{f}$, and Q_{tot}^{f} are the partial or total quantum yields of the probe in PDPC (pure fluid phase) at the temperature of the measurement. $Q_{\parallel}^{s}$, $Q_{\perp}^{s}$, and Q_{tot}^{s} are the partial or total quantum yields of the probe in DPPC (pure solid phase) at the same temperature. Q_{tot}^{mix} is, experimentally, the total quantum yield of the DPPC/PDPC mixture. It is a weighted average of the probe quantum yield in each environment multiplied by the fraction of probe molecules in each environment:

$$Q_{tot}^{mix} = Q_{tot}^{s}X_s^{p} + Q_{tot}^{f}X_f^{p} \quad (5)$$

Similarly P^{mix} is the experimental polarization of the DPPC/PDPC mixture at a particular temperature:

$$P^{mix} = Q_{\parallel}^{mix}/Q_{\perp}^{mix} = (Q_{\parallel}^{s}X_s^{p} + Q_{\parallel}^{f}X_f^{p})/(Q_{\perp}^{s}X_s^{p} + Q_{\perp}^{f}X_f^{p}) \quad (6)$$

Values of X_f and X_s at each temperature for each DPPC/PDPC mixture were derived from the complete phase diagram constructed by Sklar et al. (1979). At low temperatures X_s approaches the mole fraction of DPPC in the sample.

Results

Fluorescence Polarization of Probes in DPPC/PDPC Mixtures. Fluorescence polarization of the parinaroyl phos-

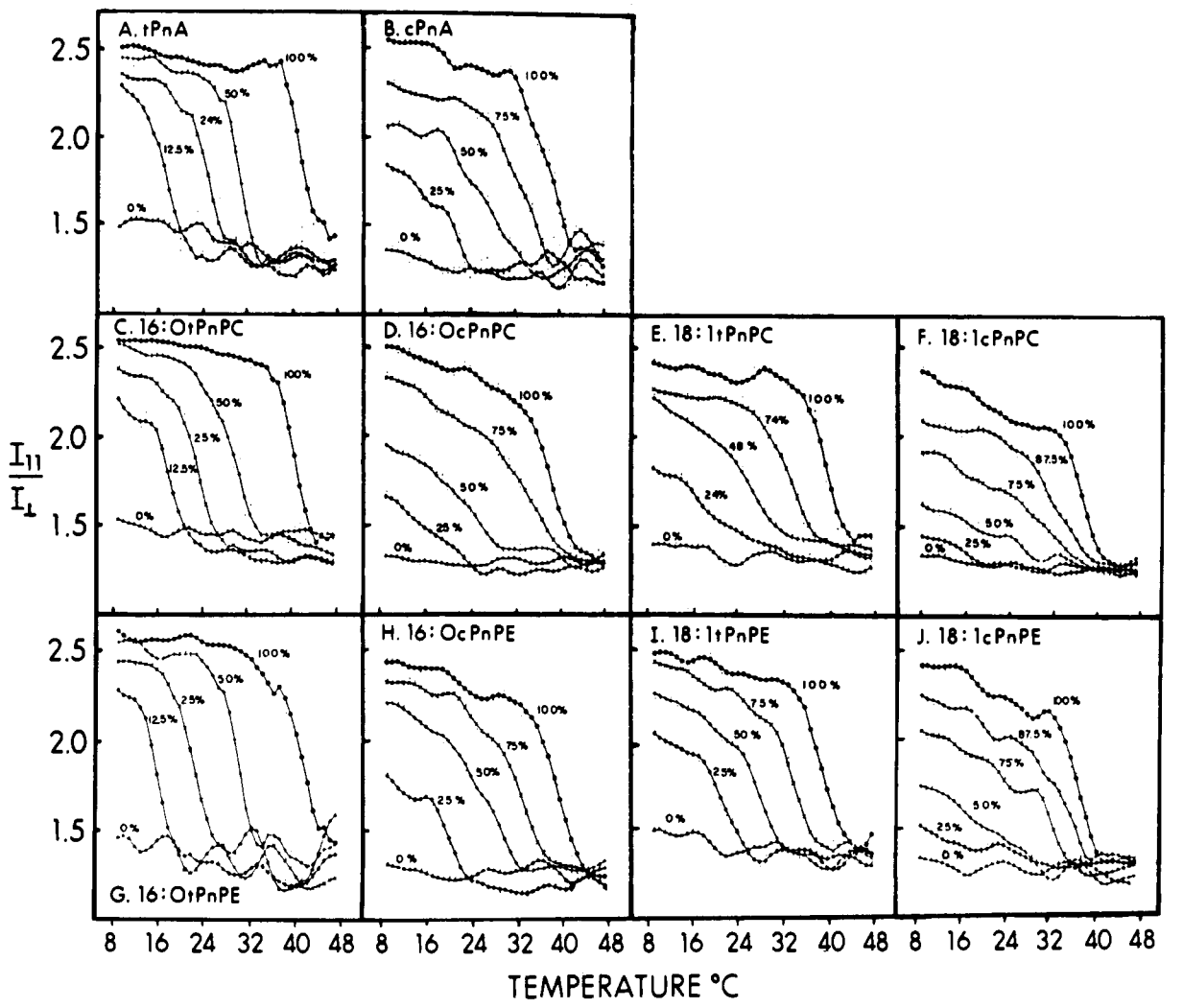

FIGURE 1: Polarization of fluorescence of probes in DPPC/PDPC mixtures vs. temperature: (A) tPnA; (B) cPnA; (C) 1-16:0,2-tPnPC; (D) 1-16:0,2-cPnPC; (E) 1-18:1,2-tPnPC; (F) 1-18:1,2-cPnPC; (G) 1-16:0,2-tPnPE; (H) 1-16:0,2-cPnPE; (I) 1-18:1,2-tPnPE; (J) 1-18:1,2-cPnPE. The numbers above the curves represent the percentage of DPPC. Vesicles were made by ethanol injection; the buffer is 115 mM NaCl/10 mM Hepes, pH 7.5.

pholipid probes as well as of *cis*- and *trans*-parinaric acids, in mixtures of DPPC and PDPC are shown in Figure 1. Phase transition onset temperatures determined from the phase diagram constructed by Sklar et al. (1979) are as follows: 12.5% DPPC, 16 °C; 24% DPPC, 23 °C; 25% DPPC, 24 °C; 48 or 50% DPPC, 32 °C; 74 or 75% DPPC, 37 °C; 87.5% DPPC, 39 °C; 100% DPPC, 41 °C. The polarization ratios of tPnA, 1-16:0,2-tPnPC, and 1-16:0,2-tPnPE reproducibly begin to increase at temperatures above the phase transition onset. This behavior has been seen previously with tPnA (Sklar et al., 1979) and is presumably due to some type of structural order on the time scale of the fluorescence lifetime of these probes.

By inspection of the plots of P vs. temperature, it is possible to see that the probes can be divided in three classes. Those that respond strongly to the presence of the solid phase are tPnA, 1-16:0,2-tPnPC, and 1-16:0,2-tPnPE, whose polarization curves in this model DPPC/PDPC system are virtually indistinguishable from one another. These three probes have low-temperature polarization ratios that are nearly as high in 50% DPPC as in pure DPPC, indicating that these probes are partitioning strongly into the solid phase in the mixture. In contrast, 1-18:1,2-cPnPC and 1-18:1,2-cPnPE show a relatively small increase in polarization ratio as the amount of solid-phase lipid is increased. The polarization of the 50% DPPC mixture is much closer to the value seen for the pure fluid phase than that for the solid phase, indicating that these probes are largely in the fluid phase. The remaining probes, cPnA, 1-18:1,2-tPnPC, 1-18:1,2-tPnPE, 1-16:0,2-cPnPC, and 1-16:0,2-cPnPE, partition more equally, as seen by their intermediate response to the level of DPPC in the mixture.

Also notable in Figure 1 is the difference in the polarization of the probes in 100% DPPC. Both 1-18:1,2-cPnPC and 1-18:1,2-cPnPE have relatively low P values well below the phase transition at 41 °C, compared to the P values obtained with, for example, cPnA. This suggests that the *cis*-parinaroyl chain in the 2 position of these probes has increased motion in gel-phase phospholipid due to the oleic acid in the 1 position.

Calculation of Partition Coefficients. Partition coefficients calculated according to eq 3 and 4 are shown in Table I. Each datum used in the calculations is the quantum yield or polarization ratio of a particular DPPC/PDPC mixture at a particular temperature. Both mean and median values are reported, since the functions do not give a Gaussian distribution of values, as well as the number of data used in the calculation. Partition coefficients determined at temperatures at which the phase composition was changing rapidly with temperature were excluded from the determination of the mean values. Also excluded from the determination of the mean values were mixtures with compositions that gave polarization ratios or quantum yields very close to those of pure DPPC or pure PDPC, as these values have more error associated with them. For example, a mixture of 75% DPPC was not included in the

Table I: Solid–Fluid-Phase Partition Coefficients[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)
tPnA	4.9 ± 2.3 (22)	6.3 ± 4.1 (22)	3.5 (80)	3.7 (25)[b]
tPnA[c]	5.1 ± 1.2	3.35 ± 0.65		
cPnA	0.68 ± 0.88 (65)	0.49 ± 0.24 (65)	0.43 (72)	0.49 (72)
cPnA[c]	0.59 ± 0.12	0.82 ± 0.33		
16:0tPnPC	4.3 ± 2.4 (23)	10.0 ± 6.4 (23)	2.5 (51)	10.0 (51)
16:0tPnPE	5.0 ± 2.5 (23)	13.8 ± 6.9 (23)	3.2 (51)[b]	9.5 (81)
18:1tPnPC	0.63 ± 0.27 (64)	0.67 ± 0.25 (64)	0.62 (71)	0.57 (71)
18:1tPnPE	0.64 ± 0.37 (62)	0.79 ± 0.17 (62)	0.54 (72)	0.76 (72)
16:0cPnPC	0.63 ± 0.25 (65)	0.61 ± 0.16 (65)	0.56 (72)	0.57 (72)
16:0cPnPE	1.01 ± 0.44 (62)	1.02 ± 0.17 (62)	0.95 (72)	1.02 (72)
18:1cPnPC	0.25 ± 0.13 (78)	0.20 ± 0.11 (58)	0.21 (102)	0.20 (102)
18:1cPnPE	0.36 ± 0.40 (77)	0.50 ± 0.54 (77)	0.31 (102)	0.28 (102)

[a] The numbers after the ± are 1 standard deviation. The numbers in parentheses are the number of data used in the calculation. [b] Data eliminated were from experiments with very high DPPC levels. [c] Sklar et al. (1979).

calculation of the mean $K_p^{s/f}$ for 1-16:0,2-tPnPC. The median values, with the two exceptions noted in the table, include all the available data. No systematic temperature dependence was seen for the partition coefficients.

Quantum yields of the phospholipid probes in fluid-phase phospholipid were similar to those of the free fatty acid probe from which they were derived. The probes with the highest $K_p^{s/f}$ values had high quantum yields in the solid phase, relative to the fluid phase, compared to the probes with lower $K_p^{s/f}$ values.

Response of Polarization Ratio to Change in Mole Fraction of the Solid Phase. In Figure 2, the relationship between P and X_s is shown for 1-16:0,2-tPnPC, 1-16:0,2-cPnPC, and 1-18:1,2-cPnPC. The curves are calculated from the rearrangement of eq 3 (Sklar et al., 1979):

$$X_s = \left[\frac{(Q_\parallel^s - P^{mix}Q_\perp^s)K_p^{s/f}}{P^{mix}Q_\perp^f - Q_\parallel^f} + 1 \right]^{-1} \quad (7)$$

They are based on the quantum yields obtained for each of the probes in pure DPPC and pure PDPC at 10 °C. The experimental polarization ratios are also for 10 °C. As is apparent in Figure 1, the polarization of 1-16:0,2-tPnPC, like tPnA and 1-16:0,2-tPnPE, is sensitive to small amounts of solid-phase lipid. The polarization of 1-16:0,2-cPnPC, like the other four probes with intermediate $K_p^{s/f}$s, responds in a nearly linear fashion to the presence of solid phase. Because of the higher quantum yield in the solid phases, the polarization ratios actually fall slightly above a straight line, although the probes prefer fluid phase slightly. On the other hand, because 1-18:1,2-cPnPC (and 1-18:1,2-cPnPE) prefer fluid phase more strongly, the polarization change in response to a phase compositional change is relatively small in the presence of small amounts of solid-phase lipid but becomes progressively greater with increasing amounts of solid phase.

Discussion

Sklar et al. (1979) showed that tPnA partitions into gel-phase lipid while cPnA prefers fluid-phase lipid slightly. *trans*-Parinaric acid's rigid chromophore geometrically resembles the chain of a saturated fatty acid in the gel state, while *cis*-parinaric more closely resembles an unsaturated fatty acid or a fluid-phase lipid with gauche conformers. Thus it is not surprising that, in the DPPC/PDPC model system, phospholipids containing a palmitic acid in the 1 position and *trans*-parinaric acid in the 2 position partition preferentially into the disaturated phospholipid gel phase. Phospholipids containing oleic and *trans*-parinaric acids or palmitic and *cis*-parinaric acids might be thought of as resembling a phospholipid with one saturated and one unsaturated chain. In the DPPC/PDPC system these probes partition equally between the two phases or prefer the fluid-phase somewhat. The 1-16:0,2-cPn probes perhaps resemble physiological molecules more than the 1-18:1,2-tPn probes, which have the *all-trans* chromophore in the 2 position. The 1-18:1,2-cPn phospholipids, resembling diunsaturated phospholipids, partition strongly into the PDPC fluid phase. This makes these probes unique among the phospholipid parinaroyl phospholipids described.

The error in determination of the partition coefficients reported in this paper is considerably larger than that reported by others using the same technique (Sklar et al., 1979). This stems from, first, our method of data collection. The present data were collected manually from the digital output of a fluorometer. This raw intensity data was used in the calculations, as indicated elsewhere in this chapter. Sklar et al. collected their fluorometer output on an XY chart recorder and then manually digitized the data. This process probably resulted in some smoothing of their data, in turn resulting in smaller standard deviations in the partition coefficients.

The second factor in our larger error is the greater loss of probe during the fluorescence temperature scans. Sklar et al. (1979) report less than a 10% loss of probe during measurements, while we typically lost about 25% of our probe. Because we used the average of the absorbances measured before and after the fluorescence measurements to calculate the quantum yields used in the partition coefficient calculations and because all the fluorescence data were taken by cooling the samples, the quantum yields of the high-temperature measurements were likely to be systematically too high while the low-temperature measurements were likely to be systematically too low. The low-temperature quantum yields obtained by cooling the samples were probably, on the average, about 15–20% (with a range of 0–35%) lower than the "true" quantum yields (compared to a standard cPnA solution). This compares to a factor of about 5 for the difference in quantum yield between solid- and fluid-phase lipid for most of the probes. This type of systematic error does not affect the calculated partition coefficients if it occurs in both the pure model lipids and in the mixtures. Because we were unable to detect any dependence of the probe loss on the model lipid in the PC system, we believe that the variation in loss of probe during measurements generated a larger degree of statistical error in the calculated partition coefficients without biasing the mean values in any particular way.

The lack of a temperature dependence for the partition coefficients of the parinaroyl phospholipids argues that the molecules are functioning as probes, not clustering, and not detecting phase transitions characteristic of themselves. This

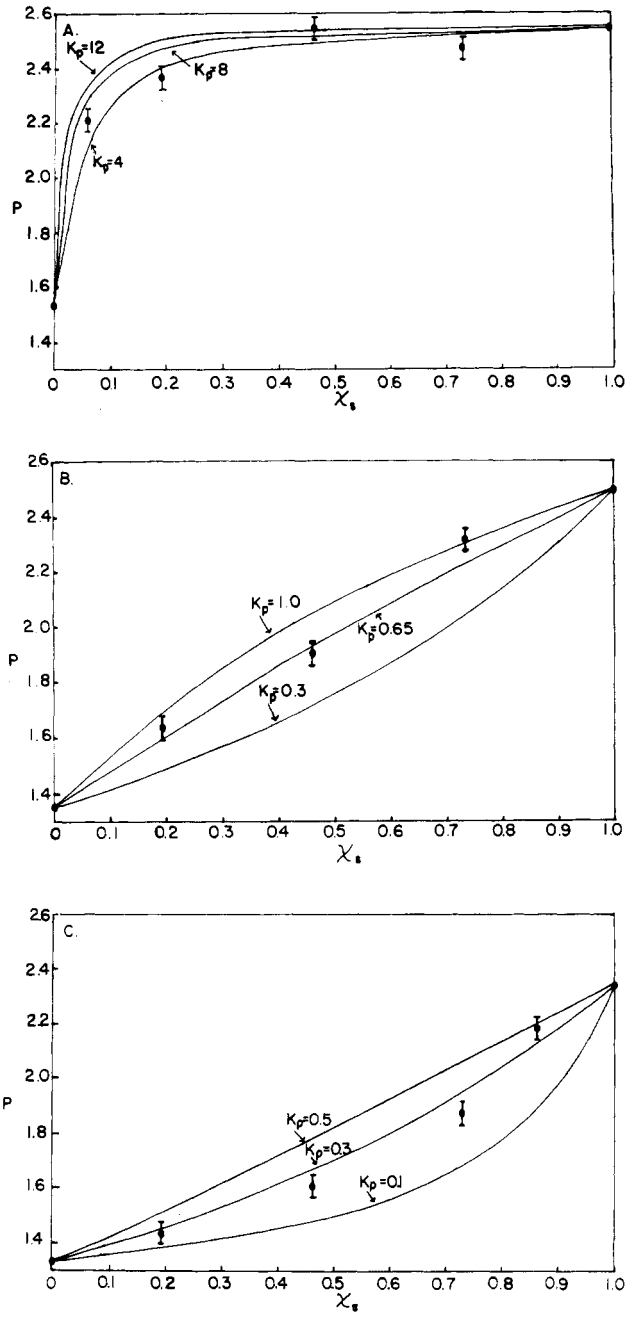

FIGURE 2: Fluorescence polarization ratio, P, vs. mole fraction of solid-phase phospholipid (X_s). Probes are (A) 1-16:0,2-tPnPC, (B) 1-16:0,2-cPnPC, and (C) 1-18:1,2-cPnPC. The data points are from polarization data (for 10 °C) such as that shown in Figure 1. The smooth curves are calculated from eq 7 for the $K_p^{s/f}$ values indicated. The partial quantum yield values at 10 °C of the probes in pure DPPC and pure PDPC used in the calculation are as follows: 1-16:0,2-tPnPC, $Q_\parallel^s = 0.187$, $Q_\perp^s = 0.073$, $Q_\parallel^f = 0.020$, $Q_\perp^f = 0.013$; 1-16:0,2-cPnPC, $Q_\parallel^s = 0.107$, $Q_\perp^s = 0.043$, $Q_\parallel^f = 0.030$, $Q_\perp^f = 0.023$; 1-18:1,2-cPnPC, $Q_\parallel^s = 0.077$, $Q_\perp^s = 0.033$, $Q_\parallel^f = 0.024$, $Q_\perp^f = 0.018$.

was of particular concern with the phospholipid probes because a species such as 1-16:0,2-cPnPC would probably undergo a gel–liquid-crystalline phase transition in the temperature range used for determination of the partition coefficients.

Phosphatidylethanolamines generally undergo solid–fluid transitions at temperatures about 20 °C above phosphatidylcholines with the corresponding acyl chains. However, acyl chains have the major effect on the partition properties of these probes in a phosphatidylcholine model system. Molecules with the phosphatidylethanolamine head group do not appear to be included in one PC phase preferentially. The following paper (Welti, 1982) examines the relative effect of head group and acyl chains in partitioning of parinaroyl phospholipids in mixed model systems of phosphatidylcholine and phosphatidylethanolamine and in biologically derived phospholipids.

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References

Ames, B. M. (1966) *Methods Enzymol. 8*, 115–118.
Azumi, T., & McGlynn, S. (1962) *J. Chem. Phys. 37*, 2413–2420.
Chadha, J. S. (1970) *Chem. Phys. Lipids 4*, 104–108.
Chen, R. F., & Bowman, R. L. (1965) *Science (Washington, D.C.) 147*, 729–732.
Christie, W. W. (1973) *Lipid Analysis*, Chapter 9, pp 279–280, Pergamon Press, Oxford.
Comfurius, P., & Zwaal, R. F. A. (1977) *Biochim. Biophys. Acta 488*, 36–42.
Gupta, C. M., Radhakrishnan, R., & Khorana, H. G. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 4315–4319.
Hubbell, W. L., & McConnell, H. M. (1969) *Proc. Natl. Acad. Sci. U.S.A. 64*, 20–27.
Kremer, J. M. H., van der Esker, M. W. J., Pathmomanohoram, C., & Wiersema, P. H. (1977) *Biochemistry 16*, 3932–3935.
Lentz, B. R., Moore, B. M., & Barrow, D. A. (1979) *Biophys. J. 25*, 489–494.
Rintoul, D. A., Sklar, L. A., & Simoni, R. D. (1978) *J. Biol. Chem. 253*, 7447–7452.
Savitzky, A., & Golay, M. J. E. (1964) *Anal. Chem. 36*, 1627–1639.
Selinger, Z., & Lapidot, Y. (1966) *J. Lipid Res. 7*, 174–175.
Sklar, L. A. (1976) Ph.D. Thesis, Stanford University, Stanford, CA.
Sklar, L. A., Hudson, B., & Simoni, R. D. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 1649–1653.
Sklar, L. A., Hudson, B. S., Petersen, M., & Diamond, J. (1977a) *Biochemistry 16*, 813–819.
Sklar, L. A., Hudson, B. S., & Simoni, R. D. (1977b) *Biochemistry 16*, 819–828.
Sklar, L. A., Miljanich, G. P., & Dratz, E. A. (1979) *Biochemistry 18*, 1707–1716.
Tecoma, E. S., Sklar, L. A., Simoni, R. D., & Hudson, B. S. (1977) *Biochemistry 16*, 829–835.
Welti, R. (1982) *Biochemistry* (following paper in this issue).