Conformation of Phosphatidylcholine in Neat and Cholesterol-Containing Liquid-Crystalline Bilayers. Application of a Novel Method†

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ABSTRACT: The conformation of phosphatidylcholine in liquid-crystalline bilayers was studied with a novel, high-resolution method employing phosphatidylcholine species containing pyrenyl moieties in both acyl chains of variable length. Analysis of the intramolecular pyrene–pyrene collision data obtained for 30 such species in terms of a simple geometrical model showed that the *sn*-1 acyl chain penetrates, on the average, 0.84 ± 0.11 methylene units (0.8 Å) deeper into the bilayer than the *sn*-2 chain at 22 °C. A similar value was obtained at 37 °C. Since the penetration difference of the *sn*-1 and *sn*-2 acyl chains is inherently coupled to the conformation of the glycerol moiety, these data mean that the glycerol moiety of phosphatidylcholine is, on the average, only moderately tilted with respect to the bilayer plane in the liquid-crystalline state. This contrasts the perpendicular orientation observed previously for phosphatidylcholine crystals [Pearson, R. H., & Pascher, I. (1979) *Nature 281*, 499–501]. Importantly, addition of 50 mol % cholesterol, which is known to reduce dramatically the interactions between phosphatidylcholine molecules in bilayers, had only a small effect on the penetration difference of the acyl chains, strongly suggesting that the conformation of phosphatidylcholine in the liquid-crystalline state is determined largely by intramolecular, rather than intermolecular, interactions.

The establishment of the conformation of glycerophospholipids in liquid-crystalline bilayers is essential for complete understanding of structural properties of these assemblies which form the "backbone" of most biological membranes. Furthermore, such information is crucial for the elucidation of the interactions of these molecules with other constituents of biological membranes such as cholesterol and proteins. Among the latter, phospholipases, many of which are intimately involved in cellular signal transduction (Billah & Anthes, 1990) and may be sensitive to the conformation of their substrate (Thuren et al., 1984), are particularly relevant in this respect.

The glycerol moiety represents the central part of glycerophospholipid molecules, and thus it is not unexpected that its conformation has received considerable attention (Seelig & Browning, 1978; Roberts et al., 1978; Zaccai et al., 1979; Seelig & Seelig, 1980; Gally et al., 1981; De Bony & Dennis, 1981; Allegrini et al., 1984; Strenk et al., 1985; Hauser et al., 1988; Han & Gross, 1990; Hubner & Mantsch, 1991; Roberts et al., 1978). Many of these studies have provided evidence for conformational inequivalency of the proximal parts of the *sn*-1 and *sn*-2 acyl chains in the liquid-crystalline state, indicating that the glycerol moiety is tilted in respect of the bilayer as has been found for phosphatidylethanolamine and phosphatidylcholine (PC)[1] crystals (Hitchcock et al., 1974; Pearson & Pascher, 1979). However, the lack of quantitative data on the acyl chain inequivalency as well as the dilemma that the molecular conformation is strongly head-group dependent in crystals (Pearson & Pascher, 1979; Pascher & Sundell, 1986; Pascher et al., 1987) but not in liquid-crystalline bilayers (Gally et al., 1981; De Bony & Dennis, 1981; Hauser et al., 1988) necessitates further studies on this matter.

We describe here a novel approach for the determination of the vertical displacement of the *sn*-1 and *sn*-2 acyl chains of PC in the liquid-crystalline state. This method makes use of PC species with a pyrenyl moiety attached to the end of both acyl chains. Determination of the intramolecular pyrene–pyrene collision frequency for several sets of such species and analysis of the data in terms of a simple geometrical model provided the value for the vertical displacement with a high (sub-angstrom) accuracy. In addition, important conclusions on the conformation of the glycerol moiety of PC in neat and cholesterol-containing bilayers could be made on the basis of the displacement data.

EXPERIMENTAL PROCEDURES

Materials. 1-Palmitoyl-2-oleoylphosphatidylcholine (POPC) and cholesterol (99+ grade) were obtained from Avanti Polar Lipids (Birmingham, Alabama) and Sigma (Deisenhofen, Germany), respectively. $Pyr_{m,n}PC$ species were synthesized and purified as described previously (Somerharju et al., 1987) Some species were further purified by reverse-phase HPLC according to Patton et al. (1982). All species were more that 98% pure as analyzed by HPLC. Phospholipase A_2 (*Naja*

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[1] Abbreviations: PC, phosphatidylcholine; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; $Pyr_{m,n}PC$, 1-pyrenyl(*m*)acyl-2-pyrenyl(*n*)-acylphosphatidylcholine, where *m* and *n* indicate the number of aliphatic carbons in the acyl chains; HPLC, high-performance liquid chromatography; *E*/*M*, excimer to monomer emission intensity ratio; MU, methylene units.

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Table I: E/M Values for $Pyr_{m,n}PC$ Subsets in POPC and POPC/Cholesterol (1:1) Matrixes

$Pyr_{m,n}PC$ (m/n)	positional purity[a]	POPC 22 °C E/M[b]	POPC 22 °C SD[c]	POPC 37 °C E/M	POPC 37 °C SD	POPC/cholesterol 22 °C E/M	POPC/cholesterol 22 °C SD	POPC/cholesterol 37 °C E/M	POPC/cholesterol 37 °C SD
$m+n=12$									
6/6	100	0.828	0.016						
4/8	83	0.720	0.024						
8/4	86	0.608	0.005						
$m+n=14$									
6/8	83	0.713	0.017	1.11	0.013	0.739	0.014	1.169	0.001
8/6	70	0.683	0.022	1.05	0.024	0.536	0.010	0.956	0.025
4/10	94	0.490	0.006	0.726	0.007	0.315	0.009	0.502	0.008
10/4	91	0.363	0.017	0.577	0.013	0.165	0.003	0.349	0.011
$m+n=16$									
8/8	100	0.686	0.010	nd[d]		0.882	0.009	1.236	0.045
6/10	85	0.600	0.013	0.924	0.003	0.724	0.006	1.122	0.047
10/6	82	0.474	0.012	0.767	0.012	0.328	0.009	0.682	0.008
4/12	96	0.329	0.006	0.496	0.023	0.156	0.004	0.267	0.018
12/4	90	0.260	0.005	0.426	0.011	0.109	0.010	0.197	0.028
$m+n=18$									
8/10	89	0.651	0.012	1.008	0.021	1.172	0.005	1.645	0.011
10/8	90	0.569	0.021	0.899	0.009	0.881	0.015	nd	
6/12	96	0.447	0.008	0.721	0.008	0.433	0.009	0.743	0.043
12/6	77	0.331	0.008	0.537	0.004	0.159	0.004	0.359	0.016
4/14	96	0.271	0.003	nd		nd		nd	
14/4	94	0.222	0.006	nd		nd		nd	
$m+n=20$									
10/10	100	0.612	0.017						
8/12	87	0.578	0.018						
12/8	87	0.466	0.005						
6/14	96	0.326	0.009						
14/6	89	0.248	0.004						
$m+n=22$									
10/12	96	0.581	0.023						
12/10	93	0.563	0.015						
8/14	83	0.464	0.011						
14/8	92	0.342	0.006						
$m+n=24$									
12/12	100	0.575	0.020						
10/14	92	0.533	0.018						
14/10	92	0.464	0.006						

[a] Acyl chain positional purity, expressed as the percentage of the nominal isomer, was determined as outlined under Experimental Procedures. [b] The E/M values were corrected for the presence of the positional isomer as described under Experimental Procedures. [c] SD, standard deviation (n = 4–9). [d] nd, not determined.

mocambique mocambique) was from Sigma. HPLC grade solvents were used throughout.

Methods. The positional distribution of the acyl chains of $Pyr_{m,n}PC$ species was determined by phospholipase A_2 hydrolysis as follows. A mixture of a pyrenyl lipid species (0.5 nmol) and egg PC (50 nmol) was taken to dryness and suspended in 300 μL of 20 mM Tris-HCl (pH 7.8) containing 6.5 mM $CaCl_2$, 2% octyl glucoside, and phospholipase A_2. The amount of enzyme was adjusted so that complete (>98%) degradation of each $Pyr_{m,n}PC$ species was obtained in 30 min at 37 °C. The fatty acids and lyso-PCs released were isolated by HPLC on a silica column (Somerharju et al., 1987) and were further fractionated into molecular species on a octadecylsilica column (Patton et al., 1982). Quantitation of the species were accomplished with a Merck-Hitachi F-1050 fluorescence detector coupled to an Merck-Hitachi F-2000 integrator, and the positional purity was calculated on the basis of the relative amounts of pyrenyl fatty acid and lyso-PC originating from the nominal compound and the isomeric impurity, respectively. Control experiments with 1-palmitoyl-2-pyrenedecanoylphosphatidylcholine and its positional isomer showed that, under the condition used, fatty acid was released only from the *sn*-2 position as expected. The positional purity of some $Pyr_{m,n}PC$s (Table I) was somewhat lower than what we have generally observed for PC synthesized with the same method (Mason et al., 1981). This appears to be due to acyl chain migration occurring during the extended phospholipase A_2 digestion required for complete cleavage of certain dipyrenylacylphosphatidylcholine precursors.

To determine the intramolecular excimer formation efficiency multilamellar liposomes containing $Pyr_{m,n}PC$ and POPC were prepared in 50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA buffer as described previously (Somerharju et al., 1985). Each sample contained 100 nmol of POPC (with or without 100 nmol cholesterol) and 0.02–0.1 nmol of a $Pyr_{m,n}PC$ species in a volume of 2 mL. After preparation, the samples were incubated at the appropriate temperature for exactly 1 h before measuring the monomer (378 nm) and excimer (475 nm) emission intensities on a Hitachi F-4000 fluorometer equipped with a cuvette holder thermostated to 22 or 37 °C and the excitation wavelength set to 344 nm. The excimer to monomer intensity ratios were then calculated after subtraction of blank values obtained with unlabeled liposomes. Finally, the ratios were corrected for the presence of the positional isomer (impurity) according to

$$E/M = [p_b E_a/M_a - (100 - p_a)E_b/M_b]/(p_a + p_b - 100) \quad (1)$$

where E/M is the corrected value and E_a/M_a and E_b/M_b are the values determined for the nominal compound and its positional isomer, respectively; p_a and p_b are the percentages of the respective compounds. It should be noted that due to

the low probe to matrix lipid ratios (2–10:10000) used E/M values were in each case essentially independent of probe concentration (SD < 2%) in accordance with Sunamoto et al. (1980). Thus only intramolecular excimer formation was measured. The independency of E/M on $Pyr_{m,n}PC$ concentration also indicates that no probe-enriched phases were present and thus the bilayer is homogenous and liquid crystalline.

THEORY

To determine the vertical displacement (d) of the *sn*-1 and *sn*-2 acyl chains, $Pyr_{m,n}PCs$ were divided into subsets so that the total number of acyl methylenes ($m + n$) was constant within each subset. This simplifies the analysis, since the number of gauche conformers should be approximately constant within a subset as deduced from the study of Mendelsohn et al. (1991). Consequently, the excimer formation efficiency within each subset should depend only on the vertical separation of the pyrenes (s) which is defined as

$$s = m - n + d \quad (m > n)$$

or

$$s = n - m - d \quad (m < n) \tag{2}$$

where m and n represent the number of methylene units in the *sn*-1 and *sn*-2 acyl chains, respectively, and d is the vertical displacement of the corresponding methylene units of the acyl chains (see Figure 1). Thus a total vertical movement equal to s is required for the apposition of the pyrenes, i.e., for the excimer formation. However, initial fitting results indicated that complete registration of the pyrenes is not required for the excimer formation and thus an additional parameter, f, representing the maximal (center to center) separation of the pyrenes allowing excimer formation, was introduced. This leads to

$$h = m - n + d - f \quad (m > n)$$

or

$$h = n - m - d - f \quad (m < n) \tag{3}$$

where h is the combined vertical movement ($= s - f$) of the pyrenes required for excimer formation. The excimer formation efficiency is assumed to be a smooth function of the equilibrium separation of the two pyrenes. This is essentially the only assumption needed to analyze the data. We tested several functions and obtained best fits with the Gaussian one:

$$E/M = v \exp(-uh^2) \tag{4}$$

where E/M is the excimer to monomer intensity ratio, v is a scaling parameter, and u relates to the vertical distribution of the pyrenes (vide infra). Initially, all parameters were allowed to vary freely to obtain f, which should be the same for all $Pyr_{m,n}PCs$ and independent of the matrix. Values ranging from 0.1 to 0.3 MU were obtained for f, depending on the $Pyr_{m,n}PC$ subset used, and the mean value of 0.2 MU was used in the final fitting to obtain d, v, and u for each subset. These latter parameters were obtained independently for each subset so that the possible chain-length (i.e., pyrene position) dependent effects could be observed.

That best fits were obtained with a Gaussian function (eq 4) strongly suggests that the vertical distribution of the pyrenes in the bilayer is approximately Gaussian. This is in accordance with the recent findings that many functional groups of phospholipid molecules obey Gaussian distribution in liquid-crystalline bilayers (White et al., 1991a,b; 1992).

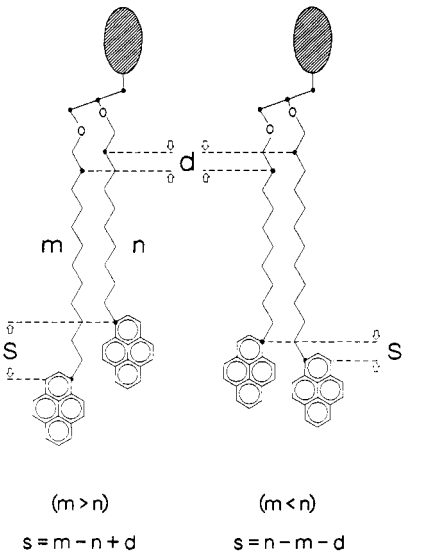

FIGURE 1: Schematic structure of $Pyr_{m,n}PC$ species and definition of the parameters used in the conformational analysis. The two lipids shown are positonal isomers, i.e., they have the same acyl residues but in reversed *sn* positions. The number of aliphatic carbons in the *sn*-1 and *sn*-2 chain is denoted by variables m and n, respectively. In the left-hand molecule m is 12 and n is 10, while in the right-hand one m and n are equal to 10 and 12, respectively. The vertical displacement of the equivalent carbon atoms of the *sn*-1 and *sn*-2 acyl chains is denoted by d. The variable s is the (acyl chain and d dependent) vertical separation of the pyrenes. Note that the algebraic definition of s depends on the relative magnitude of m and n.

The $1/e$ width for the vertical (Gaussian) distribution of the pyrenes in the bilayer was calculated from (see Appendix)

$$(1/e)_{pyr} = 1/\sqrt{2u} \tag{5}$$

RESULTS

To obtain quantitative information on the penetration difference of the acyl chains of phosphatidylcholine in the liquid-crystalline state, a series of species ($Pyr_{m,n}PCs$) with a pyrenyl moiety attached to the end of the *sn*-1 and *sn*-2 acyl chains (Figure 1) was synthesized. The lengths of *sn*-1 and *sn*-2 acyl chains was varied independently from 4 to 14 carbons, giving a total of 30 distinct molecular species (Table I). These species were incorporated in trace amounts into multilamellar liposomes consisting of 1-palmitoyl-2-oleoyl-phosphatidylcholine (POPC), and the ratio of the pyrene excimer to monomer emission intensities (E/M) was determined at 22 and 37 °C. Due to low probe concentrations used, the measured E/M values are directly proportional to the *intramolecular* pyrene–pyrene collision frequency (see Methods). This frequency depends on the average distance between the two pyrenyl moieties and thus provides information on the average vertical displacement (d) of the *sn*-1 and *sn*-2 acyl chains. Furthermore, since this displacement is coupled to the orientation of the glycerol moiety (Figure 1), measurement of d also allows estimation of the orientation of this moiety relative to the membrane surface (see Discussion).

As shown in Table I, the E/M values obtained for the $Pyr_{m,n}PC$ species containing the longer pyrenyl chain in the *sn*-2 position ($P_{6,10}PC$, for instance) were, without exceptions, higher that those obtained for their positional isomers (i.e., $P_{10,6}PC$). The only feasible explanation for this is that the *sn*-1 chain penetrates deeper into the bilayer than the *sn*-2 chain, which is in agreement with previous conclusions (Seelig & Seelig, 1980; Gally et al., 1981; Han & Gross, 1990; Hübner & Mantsch, 1991).

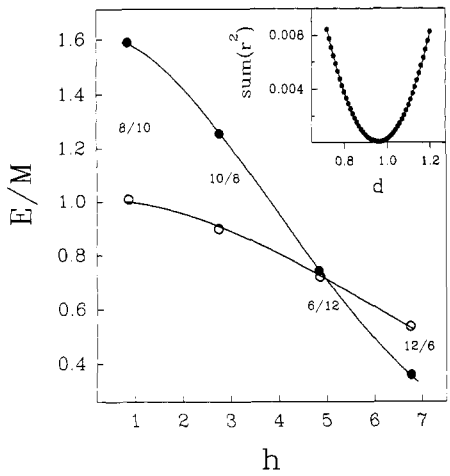

FIGURE 2: Determination of the vertical displacement (d) of the sn-1 and sn-2 acyl chains by curve fitting. A Gaussian function $E/M = v\exp(-uh^{-2})$ was fitted to the E/M values determined for the 18-subset in POPC (open symbols) or POPC/cholesterol (1:1 mole ratio, closed symbols) matrix at 37 °C to obtain the value of d. See Theory for details and for the definition of h and other parameters. Error bars have been omitted from the data points as they are generally smaller than the symbols (see Table I). The inset demonstrates the sensitivity of the fit quality to the chosen value of d (POPC matrix). sum(r^2) is the fitting error.

A simple geometrical model was developed to obtain d, the vertical displacement of the sn-1 and sn-2 acyl chains (see Theory). To simplify the analysis, $Pyr_{m,n}PC$ species were divided into subsets so that the number of acyl carbons ($m + n$) was constant (12, 14, 16, etc.) in each subset. The parameter d was then obtained by fitting a Gaussian equation to the measured E/M values (Figure 2). In general, good fits were obtained, and the d values could be determined with relatively high precision (Figure 2 and Table II). In neat POPC matrix, d varied from 0.7 to 0.98 methylene units (MU), the mean value being 0.84 ± 0.11 MU. On the basis of the previously established (McIntosh & Holloway, 1987) effective length of a methylene unit, this corresponds to approximately 0.75 Å. Parameter d was determined for some subsets also at 37 °C (Table II). The average value (0.71 ± 0.2 MU) obtained is not statistically different from that obtained at 22 °C, suggesting that the conformation of PC is not significantly temperature dependent in the liquid-crystalline state.

The parameter d was determined for a limited number of $Pyr_{m,n}PC$ subsets also for cholesterol/POPC (1:1 molar ratio) bilayers. Although cholesterol markedly influenced the absolute E/M values (Table I and Figure 2) as expected on the basis on its dramatic effects on phospholipid acyl chain conformation (Ghosh & Seelig, 1982; Mendelsohn et al., 1991), it had, on the average, only a modest effect on the value of d both at 22 and 37 °C (Table II). Thus cholesterol does not seem to influence markedly the penetration difference of the sn-1 and sn-2 acyl chains of the PC in the liquid-crystalline state.

DISCUSSION

While several studies have provided evidence for the conformational inequivalency of the sn-1 and sn-2 acyl chains of glycerophospholipids in the liquid-crystalline state (Seelig & Seelig, 1975; Seelig & Browning, 1978; Roberts et al., 1978; Gally et al., 1981; De Bony & Dennis, 1981; Allegrini et al., 1984; Han & Gross, 1990; Hubner & Mantsch, 1991), quantitative determination of the vertical displacement of the acyl chains has not, to the best of our knowledge, yet been achieved. Approaching this, Wiener and White (1991a,b) have recently obtained diffraction data on oriented, liquid-crystalline PC bilayers (at 66% relative humidity), which indicated that the vertical displacement of the sn-1 and sn-2 chains should be less than 1 Å. The method used did not, however, allow these authors to obtain a definite value (or sign) for the displacement. Thus our present result, i.e., that the sn-1 chain of $Pyr_{m,n}PC$s peneterates, on the average, 0.84 ± 0.11 MU (approximately 0.75 Å) deeper into to the bilayer than the sn-2 chain, appears to represent the first measurement of the vertical displacement of the acyl chains in the liquid-crystalline state.

A simple empirical model was used to obtain the conformational parameter d. The model assumes that the (intramolecular) excimer is formed mostly by those molecules which have their acyl chains in all-trans conformation (Figure 1). The validity of this assumption is strongly supported by simple geometrical considerations indicating that only in rare cases are the positions of the gauche bonds and the orientation of the chains segments in gauche conformers such that the pyrenes are positioned in a manner allowing excimer formation. That excimers are mainly formed by all-trans conformers is also

Table II: Conformational Parameters for $Pyr_{m,n}PC$ Subsets in POPC and POPC/Cholesterol Bilayers[a]

$Pyr_{m,n}PC$ $m+n$	POPC		POPC/cholesterol	
	22 °C	37 °C	22 °C	37 °C
12				
d	0.70			
v	0.84			
u	0.016			
$1/e$	5.6			
Σr^2	1.9×10^{-13}			
14				
d	0.70	0.57	1.0	0.66
v	0.74	1.2	0.74	1.2
u	0.016	0.017	0.036	0.031
$1/e$	5.6	5.4	3.7	4.0
Σr^2	4.8×10^{-4}	6.7×10^{-5}	1.4×10^{-3}	2.6×10^{-3}
16				
d	0.78	0.63	1.2	0.99
v	0.68	1.0	0.95	1.3
u	0.014	0.013	0.040	0.029
$1/e$	6.0	6.0	3.5	4.2
Σr^2	1.7×10^{-3}	5.7×10^{-3}	5.7×10^{-3}	1.5×10^{-2}
18				
d	0.92	0.94	0.93	0.96
v	0.66	1.0	1.2	1.6
u	0.014	0.014	0.044	0.033
$1/e$	6.0	6.0	3.4	3.9
Σr^2	1.2×10^{-2}	1.9×10^{-4}	1.1×10^{-4}	6×10^{-5}
20				
d	0.87			
v	0.63			
u	0.013			
$1/e$	6.2			
Σr^2	6×10^{-4}			
22				
d	0.98			
v	0.60			
u	0.012			
$1/e$	6.4			
Σr^2	5.2×10^{-4}			
24				
d	0.90			
v	0.58			
u	0.010			
$1/e$	7.1			
Σr^2	1.7×10^{-12}			
d_{mean} ± SD	0.84 ± 0.11	0.71 ± 0.20	1.0 ± 0.14	0.87 ± 0.18

[a] The values for d, v, and u were obtained by parameter fitting as described under Theory. The halfwidth of the vertical distribution of the pyrenes, $1/e$, was derived from u (see Appendix). Σr^2 is the fitting error.

indicated by the marked decrease in E/M when the length of the acyl chains of homoacyl $Pyr_{m,n}PCs$ increases from 6 to 12 methylene units (Table I) despite that the vertical pyrene–pyrene distance (determined by d) should remain constant. The most feasible explanation for such a decrease in that the fraction of all-trans conformers decreases when the number of gauche bonds in the acyl chains increases with chain length (Casal & McElhaney, 1990). Furthermore, even among the gauche conformers, the probability of excimer formation depends qualitatively similarly on d as with all-trans conformers. However, the average vertical interpyrene distance is somewhat smaller than in the all-trans conformers, indicating that our model may slightly underestimate the value of d.

Obviously, the values obtained for d in this study are directly valid only for dipyrenylphosphatidylcholines. However, they are valid also for natural (unlabeled) phosphatidylcholines if the vertical distribution of the pyrenyl moieties in the bilayers is not significantly constrained by pyrene–matrix or pyrene–pyrene interactions. Several lines of evidence provide support for such unconstrained vertical distribution of the pyrenyl moieties. First of all, the similarity of the d values obtained with seven independent sets of $Pyr_{m,n}PC$ species with the average acyl chain length varying from 6 to 12 carbons strongly suggests that the host lipid matrix does not impose marked constraints on the localization of the pyrenyl moieties. Second, best fits to the data were obtained when a Gaussian function (eq 4) was applied (see Theory). Third, the average of the $1/e$ width for the vertical distribution of the pyrenes in POPC at 22 °C was 6.1 MU (Table II), which corresponds to approximately 5.5 Å (McIntosh & Holloway, 1987). This is similar to the values of 5 ± 0.6 Å and 4.29 ± 0.16 Å determined for a brominated and deuteriated C9–C10 double bond, respectively (Wiener & White, 1991a,b). Fourth, we have recently found, by using independent methods, that the mean vertical position of the pyrenyl moiety of a monopyrenyl PC in the bilayer is a smooth function of the length of the labeled acyl chain (manuscript in preparation). Similar results have been obtained for pyrenyl fatty acids (Luisetti et al., 1979). Fifth, the possibility that long-range attractive forces between the excited and the ground-state pyrenes would significantly distort the conformation of the acyl chains is excluded by the finding that good fits to the data could obtained only when a critical value equal or below 0.3 MU was used for the vertical pyrene–pyrene separation (i.e., f; see Theory). Finally, we note that the present results are in agreement with previous (qualitative) data, obtained with various techniques, indicating deeper penetration of the *sn*-1 acyl chain of phosphatidylcholines in liquid-crystalline bilayers (see above).

The reason for the somewhat lower d values obtained for the short-chain $Pyr_{m,n}PCs$ (Table II) is not clear. Although they may relate properties peculiar to these species (i.e., perturbation by the pyrenes), an intriguing alternative is that d is not constant for all PC species but is influenced by the structure of acyl chains. In this respect, it is relevant to note that polyunsaturated acyl chains seem to fold in a manner which makes them considerably bulkier than the more saturated ones (Applegate & Glomset, 1986) and thus possibly sterically similar to the short chain pyrenyl fatty acids. Supporting such similarity, we have recently found that short-chain pyrenyl fatty acids incorporate exclusively to the *sn*-2 position of phospholipids of BHK cells (Kasurinen & Somerharju, 1991), a behavior generally observed for polyunsaturated natural fatty acids (Holub & Kuksis, 1978). The long-chain pyrenyl fatty acids, on the other hand, incorporated preferentially into the *sn*-1 position, thus resembling saturated natural fatty acids (Holub & Kuksis, 1978).

Because of an inherent conformational coupling, important conclusions on the orientation of the glycerol moiety can be made on the basis of acyl chain displacement data obtained in this study. Hauser et al. (1988) have suggested that the conformation of glycerophospholipid molecules in liquid-crystalline bilayers fluctuates between four minimum-energy states. Although the present steady-state fluorescence measurements do not allow the determination the probabilities of the individual states, comparison of the d values (Table II) with those derived from the structures given by Hauser et al. indicate that the time-averaged conformation of PC in the liquid-crystalline state is similar to the conformation B_v [see Hauser et al. (1988)] where the glycerol moiety is slightly tilted in respect of the bilayer plane and the *sn*-1 penetrates approximately one methylene unit deeper into the bilayer than the *sn*-2 chain.

Such a conformation is quite different from the one found for PC crystals, where there is an approximately 3 MU displacement of the acyl chains and the glycerol moiety lies almost parallel to the long axis of the molecule (Pearson & Pascher, 1979). The conformation in the crystal obviously reflects the adaptation of the glycerol moiety and the all-trans acyl chains to match the (relatively) large cross-sectional area of the phosphocholine head group (Gennis, 1989). In the liquid-crystalline state there is no (or little) need for such an adaptation, because the cross-sectional area of the melted acyl chains is similar to that of the head group (Gennis, 1989). Addition of 50 mol % cholesterol had only a modest effect on the average value of d (Table II); despite that, the presence of the cholesterol molecules should lead to a dramatic decrease in the interactions between the phospholipid molecules (Presti et al., 1982; Singer & Finegold, 1990). This result strongly suggests that the conformation in the liquid-crystalline state is determined largely by intramolecular, rather than intermolecular, interactions. In line with this conclusion, we have found that the vertical displacement of the acyl chains of $Pyr_{m,n}PCs$ in detergent (E_8C_{12}) micelles, i.e., a completely different matrix, is similar to that found in POPC (unpublished data). Previously, Dennis and co-workers have provided evidence for a similar average conformation of glycerophospholipids in bilayers and micelles (Roberts et al., 1978; De Bony & Dennis, 1981). A tendency for optimal intramolecular chain stacking could be the critical factor in determining the conformation of phospholipids in liquid-crystalline bilayers, as has been suggested by others (McAlister et al., 1973; Hauser et al., 1988).

The orientation of the glycerol moiety of PC almost parallel to the bilayer plane in the liquid-crystalline state is expected to increase the elasticity of the bilayer because pressure-induced reorientation to the perpendicular conformation and concomitant reduction of surface area is possible. This may be particularly important for the function of pumps and other membrane proteins undergoing conformational changes involving surface area fluctuations.

The high resolution of the method described here makes it a promising tool for further studies on lipid conformation. In particular, time-resolved measurement of the intramolecular excimer formation should provide important new information on the relative occupancy of different conformational states as well as on the dynamics of the glycerol moiety and the acyl chains.

APPENDIX

The above data provided information on the equilibrium positions of the pyrenes relative to each other. The *vertical*

distribution of the pyrenes around these equilibrium positions can be estimated for each $Pyr_{m,n}PC$ subset from the distribution of excimer formation as follows. First, assuming that both pyrenes obey Gaussian distribution, the local concentrations of the two pyrenes along the bilayer normal (C_{pyr_1}, C_{pyr_2}) are

$$C_{pyr_1} = v'e^{-u'(y-a)^2}$$

$$C_{pyr_2} = v'e^{-u'(y-b)^2} \quad \text{(A1)}$$

where the variable y is the distance from the bilayer surface and the parameters a and b indicate the surface to (Gaussian) peak distances for the shallower (pyr_1) and deeper pyrene (pyr_2), respectively. The probability of excimer formation [$E(y)$] at distance y is proportional to the product of the pyrene concentrations:

$$E(y) \approx C_{pyr_1}C_{pyr_2} = v'^2e^{-u'[(y_1-a)^2+(y_2-b)^2]} \quad \text{(A2)}$$

Excimer formation is possible when the vertical separation of the pyrenes is $y_1 - y_2 = f$, where f is a constant (see Theory). We denote $y = y_1$ and $h = s - f$, and because $a = b + s$ (where s is the peak to peak separation of the pyr_1 and pyr_2 Gaussians), eq A2 transforms to eq A3 and then to eq A4:

$$E(y) \approx v'^2e^{-u'[(y-a)^2+(y-a+h)^2]} \quad \text{(A3)}$$

$$E(y) \approx v'^2e^{-2u'[[y-a+(h/2)]^2+[(h/2)]^2]} \quad \text{(A4)}$$

Integration over the bilayer gives

$$E \approx \int E(y)\,dy = v'^2e^{-2u'(h/2)^2}\int e^{-2u'[y-a+(h/2)]^2} \quad \text{(A5)}$$

This integral can be approximated:

$$\int e^{-2u'[y-a+(h/2)]^2}\,dy \simeq \sqrt{\pi}/\sqrt{2u'} \quad \text{(A6)}$$

which is exact if the integration is over the whole y axis. Because this integral is part of the scaling factor, it is not important regarding the final conclusions. By denoting

$$v = v'^2\frac{\sqrt{\pi}}{\sqrt{2u'}}; \qquad u = \frac{u'}{2} \quad \text{(A7)}$$

where v and u are constants typical for each $Pyr_{m,n}PC$ subset, we obtain

$$E = ve^{-uh^2} \quad \text{(A8)}$$

Finally, the half-widths for the excimer formation $(1/e)_{exc}$ and pyrene distribution $(1/e)_{pyr}$ are

$$\left(\frac{1}{e}\right)_{exc} = \frac{1}{\sqrt{u}}; \qquad \left(\frac{1}{e}\right)_{pyr} = \frac{1}{\sqrt{2u}} \quad \text{(A9)}$$

Thus $(1/e)_{exc}$ has to be divided by $\sqrt{2}$ to obtain $(1/e)_{pyr}$. This is because the movement of each pyrene required for excimer formation is only half of the equilibrium distance of the pyrenes.

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