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Membrane penetration depth and lipid phase preference of acyl-labeled dansyl phosphatidylcholines in phosphatidylcholine vesicles

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Abstract

The dansyl fluors of 1-oleoyl-2-[4-(dansyl)amino]butyl]-*sn*-glycero-3-phosphocholine (18:1,4-dansyl PC) and 1-palmitoyl-2-[11-[(dansyl)amino)]undecanoyl]-*sn*-glycero-3-phosphocholine (16:0,11-dansyl PC) were shown to reside at similar depths in phosphatidylcholine vesicles at pH 7. Analysis of fluorescence emission maxima showed that the dansyl groups of both 18:1,4-dansyl PC and 16:0,11-dansyl PC in phosphatidylcholine vesicles experienced environments more polar than methanol, suggesting that the dansyl group attached to the terminus of the undecanoyl chain must fold back toward the bilayer surface. Fluorescence polarization measurements in solid/fluid lipid mixtures show that both probes partition strongly into fluid phase lipid. The very low polarization values of 16:0,11-dansyl PC in lipid vesicles are consistent with the notion that the dansyl fluor of 16:0,11-dansyl PC existed in an environment allowing a high degree of motional freedom due to folding back of the dansyl group attached to the undecanoyl chain. © 1997 Elsevier Science B.V.

Keywords: Dansyl phosphatidylcholine; Spin-labeled lipid; Lipid phase; Parallax analysis; Steady-state fluorescence polarization; Fluorescence emission maximum

Abbreviations: Dansyl, 5-dimethylaminonaphthalene-1-sulfonyl; 18:1,4-dansyl PC, 1-oleoyl-2-[4-(dansyl)amino]butyl]-*sn*-glycero-3-phosphocholine; 16:0,11-dansyl PC, 1-palmitoyl-2-[11-[(dansyl)amino)]undecanoyl]-*sn*-glycero-3-phosphocholine; Dansyl-PE, *N*-(dansyl)dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; PC, 1,2-acyl-*sn*-glycero-3-phosphocholine or phosphatidylcholine; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DLiPC, 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine; NBD, 7-nitrobenz-2-oxa-1,3-diazol-4-yl; NBD-PE, *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; 16:0,6-NBD PC, 1-palmitoyl-[2-[(6-NBD)amino]caproyl]-*sn*-glycero-3-phosphocholine; 16:0,12-NBD PC, 1-palmitoyl-[2-[(12-NBD)amino]docanoyl]-*sn*-glycero-3-phosphocholine; 5-SLPC, 1-palmitoyl-2-(5-doxyl)stearoyl-*sn*-glycero-3-phosphocholine; 10-SLPC, 1-palmitoyl-2-(10-doxyl)stearoyl-*sn*-glycero-3-phosphocholine; 12-SLPC, 1-palmitoyl-2-(12-doxyl)stearoyl-*sn*-glycero-3-phosphocholine; Tempo-PC, 1,2-dioleoyl-*sn*-glycero-3-phosphotempocholine

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1. Introduction

Dansyl probes were introduced by Weber [1]. The dansyl moiety has been widely used for fluorescently labeling proteins. Dansyl is an especially useful fluor in this regard, since the excitation spectrum of the dansyl group allows it to serve as an energy transfer acceptor for tryptophan fluorescence.

Dansyl-labeled lipids also have been used to study membrane properties. Waggoner and Stryer [2] examined the location of the dansyl group of head group-labeled phosphatidylethanolamine in a lipid bilayer by comparing the position of the fluorescence emission maxima in various solvents to the position of the maximum in lipid vesicles. Their data indicated that fluor attached to the head group in dansyl-PE in phosphatidylcholine bilayers was in an environment with a polarity similar to methanol. This was interpreted as indicating that the dansyl group was located in the region of the bilayer near the phosphatidylcholine glycerol backbones. In the same study, the polarity of the location in bilayers of the short chain dansyl derivative *N*-dansylethylamine also was examined. Like dansyl-PE, this compound exhibited an emission maximum similar to methanol, implying that it is located in the same area of the bilayer as the dansyl group on head group-labeled phosphatidylethanolamine. This result suggested that the dansyl chromophore itself, rather than its attachment site, might play a major role in determining location in the lipid bilayer.

In the work presented here, we have examined the location in a phosphatidylcholine bilayer of the dansyl fluor attached to phosphatidylcholine fatty acyl chains four and eleven carbon long. We demonstrate that, in each case, the dansyl chromatophores resided in a polar region of the bilayer.

2. Material and methods

2.1. Materials

5-SLPC[1], 10-SLPC, 12-SLPC, NBD-PE, DOPC, and lyso PCs were obtained from Avanti Polar Lipids. 4-[(Dansyl)amino]butanoic acid was from Chemical Dynamics Corp. (South Plainfield, NJ). 11-[(Dansyl)amino]undecanoic acid was from Molecular Probes.

18:1,4-Dansyl PC and 16:0,11-dansyl PC were synthesized by a modification of the synthesis of fatty acid anhydrides by Selinger and Lapidot [3] and the acylation procedure of Gupta et al. [4]. Each fatty acid was dissolved (30 mg/ml) in chloroform which had been redistilled from phosphorus pentoxide. An equimolar amount of dicyclohexylcarbodiimide was added and the reaction forming the fatty acyl anhydrides was allowed to proceed in the dark for 2 h. The reaction mixture was filtered through chloroform-washed glass wool. The solvent was removed from the filtrate under vacuum with a rotary evaporator. The fatty acyl anhydrides were redissolved in redistilled chloroform (15 mg/ml) and added in 1.5-fold excess (3-fold excess of original fatty acids) to 1-fatty acyl,2-lyso PC and dimethylaminopyridine (equimolar to the lyso PC), which had been dried together by repeated evaporation of dry toluene from the mixture. The acylation reaction was allowed to proceed under nitrogen overnight in the dark. The phosphatidylcholines were purified by preparative TLC in chloroform/methanol/water (65:25:4, v/v) to yield a single fluorescent spot with an R_f slightly higher than that of dipalmitoylphosphatidylcholine. The concentration of the dansyl PCs was determined by absorbance, assuming an molar extinction coefficient of 4400 M^{-1} cm^{-1} in methanol, and by phosphate assay [5]. The fluor to phosphate ratio in each case was between 0.8 and 1.0.

2.2. Parallax analysis of membrane penetration depth

Multilamellar dispersions containing 1.6 nmol of one dansyl PC and 160 nmol DOPC were prepared as described by Chattopadhyay and London [6], except that the samples were dispersed in 3 ml, rather than 1.5 ml, of 150 mM sodium chloride, 10 mM sodium phosphate, pH 7.0. Fluorescence was measured at room temperature in a SLM Model 8000 fluorimeter. Background intensity from samples without fluor was subtracted from the intensity in the presence of the fluor. Dansyl excitation was at 335 nm and emission was measured at 516 nm. NBD excitation was at 469 nm and emission was measured at 533 nm. The emission and excitation slits had a nominal bandpass of 4 nm.

The concentration of each spin-labeled phosphatidylcholine was determined by measuring the quenching of NBD-PE by the spin-labeled PC [7]. When $\ln(F/F_0)$ (F, quenched fluorescence; F_0, unquenched fluorescence) was plotted vs. the mole fraction spin-label, the slopes were found to be very linear (correlation coefficient $r = 0.999$) at spin-label concentrations less than 50 mol%. The spin-label concentrations were determined by setting the slopes of the lines obtained at low spin-label concentration to -4.43 for 5-SLPC, -3.17 for 12-SLPC, and -3.89 for 10-SLPC. The value of the slope for 10-SLPC was obtained from the published values of the slopes for 5-SLPC and 12-SLPC by noting equation 4 of Chattopadhyay and London [6]

$$F/F_0 = e^{-\pi R_c^2 C + \pi z^2 C} \quad (1)$$

where R_c is the radius around a quencher within which fluorescence in quenched completely, z is the vertical distance between the quencher and fluor, and C is the mole fraction of quencher (spin-label) in the total lipid/70 Å^2, the surface area of one phosphatidylcholine molecule. From this equation, it can be seen that the slope of a plot of $\ln(F/F_0)$ vs. the mole fraction spin-label equals $[\pi(z^2 - R_c^2)]/70$ Å. Substituting $z_{cF} - 12.15$ Å for z for the 5-SLPC and $z_{cF} - 5.85$ Å for z for 12-SLPC and using the known slopes, we solved for z_{cF}, the distance from the center of the bilayer to the fluor and R_c. For NBD-PE, z_{cF} was 11.2 Å, and R_c was 9.97 Å. Assuming that $z = z_{cF} - 7.65$ Å, the slope of $\ln(F/F_0)$ vs. the mole fraction of 10-SLPC was calculated to be -3.89. The concentrations of the spin labels were determined to be 5-SLPC, 96.3%; 10-SLPC, 94.1%; and 12-SLPC, 78.2%.

The distance of each dansyl fluor from the bilayer center, z_{cF}, was calculated using equations 8 and 9 of Chattopadhyay and London [6]. Since the fluorescence was measured on samples with varying actual spin-label concentrations, F/F_0 values for samples with one quencher in a pair were obtained at the actual spin-label concentrations of the other quencher in the pair by linear interpolation between the closest two points on a plot of $\ln(F/F_0)$ vs. the spin-label concentration of the first quencher. R_c was calculated from Eq. (1) (Eq. 4 of Ref. [6]).

2.3. Fluorescence emission maxima

The fluorescence emission maxima were determined on a Spex Fluorolog fluorimeter using 0.9 μM solutions in various solvents with excitation at 335 nm. Analysis of the emission peak position was by comparison of each fluor with the same compound in solvents [2]. For measurement of the fluorescence emission maxima in DOPC vesicles, vesicles were formed by ethanol injection. DOPC (980 nmol) and each fluor (20 nmol) were dissolved in ethanol at a total lipid concentration of 1.7 mM. The ethanolic solution (30 μl) was rapidly injected with vortexing into 3 ml 150 mM sodium chloride, 10 mM sodium phosphate, pH 7.0, yielding a 17 μM dispersion, containing 1% ethanol.

2.4. Fluorescence polarization and partition coefficients

Steady-state fluorescence polarization measurements were performed on an SLM Model 8000 fluorimeter, operating in the L-format with corrections made for dark current. Measurements were performed at 5°C, allowing a 5 min equilibration time before recording the intensities from each sample. Alignment of the polarizers was verified with 0.5 μM carboxyfluorescein in 95% glycerol which, at 5°C, gave a polarization ratio ($I_{\parallel}/I_{\perp}$) of 2.93 (theoretical maximum = 3).

Ethanol injection vesicles for these experiments were prepared by injection of 48 μl of an ethanolic solution containing 1 nmol dansyl PC and 800 nmol DPPC and/or DLiPC into 3.2 ml 115 mM NaCl, 1 mM EDTA, 10 mM Hepes, pH 7.5, yielding a 0.25 mM lipid dispersion containing 1.5% ethanol. Comparison of polarization values obtained on dispersions ranging in concentration from 0.1 to 2 mM lipid demonstrated that there was no effect of lipid concentration on polarization values. Removal of ethanol by dialysis produced a small, but significant increase in polarization of 0.015 to 0.030. This is equivalent to an increase of 0.05 to 0.10 in the polarization ratio ($I_{\parallel}/I_{\perp}$). Ethanol was not routinely removed.

The solid–fluid partition coefficient is defined as

$$K_p^{s/f} = (X_s^p/X_s)/(X_f^p/X_f) \quad (2)$$

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 fluorescent probe in the fluid and solid phases [8]. The solid–fluid partition coefficient of each fluor was calculated from the partial intensities as

$$K_p^{s/f} = \left(P^{mix} I_\perp^f - I_\parallel^f\right) X_f / \left[\left(I_\parallel^s - P^{mix} I_\perp^s\right) X_s\right] \qquad (3)$$

where $I_\parallel^f$ and $I_\perp^f$ are the partial intensities of the fluor in pure DLiPC (pure fluid phase) and $I_\parallel^s$ and $I_\perp^s$ are the partial intensities of the fluor in DPPC (pure solid phase). P^{mix} is equal to $I_\parallel/I_\perp$, the experimental polarization ratio of each DPPC/DLiPC mixture. X_f is the mole fraction of the lipid mixture in the fluid phase and was calculated from the phase diagram for the DPPC/DLiPC mixture constructed by Martin et al. [9]. Eq. 4 is identical to the equation developed by Sklar et al. [8], except that partial intensities have been substituted for partial quantum yields. This substitution is justified, assuming the absorbance of each sample is the same. Since the absorbances of fluor in our lipid dispersions were very low ($A_{335} \approx 0.0014$) and the same amount of fluor was added to each sample, we have made this approximation.

3. Results

Fig. 1, Panel A shows the fluorescence of 16:0,11-dansyl PC as a function of spin-label concentration. Of the spin-labels examined, the 5-SLPC is most effective at quenching the dansyl fluorescence, while the 10-SLPC is second most effective, and the 12-SLPC is least effective. These data suggest that the fluor is closest to the quencher in 5-SLPC. Analysis of these data by the parallax method [6] shows that the apparent position of the dansyl group is about 10 Å from the center of the bilayer (Table 1). Fig. 1, Panel B shows similar data for 18:1,4-dansyl PC. The fluor of this dansyl PC was quenched to a similar extent by 5-SLPC and 10-SLPC, but quenched to a lesser extent by 12-SLPC. Calculation of the location

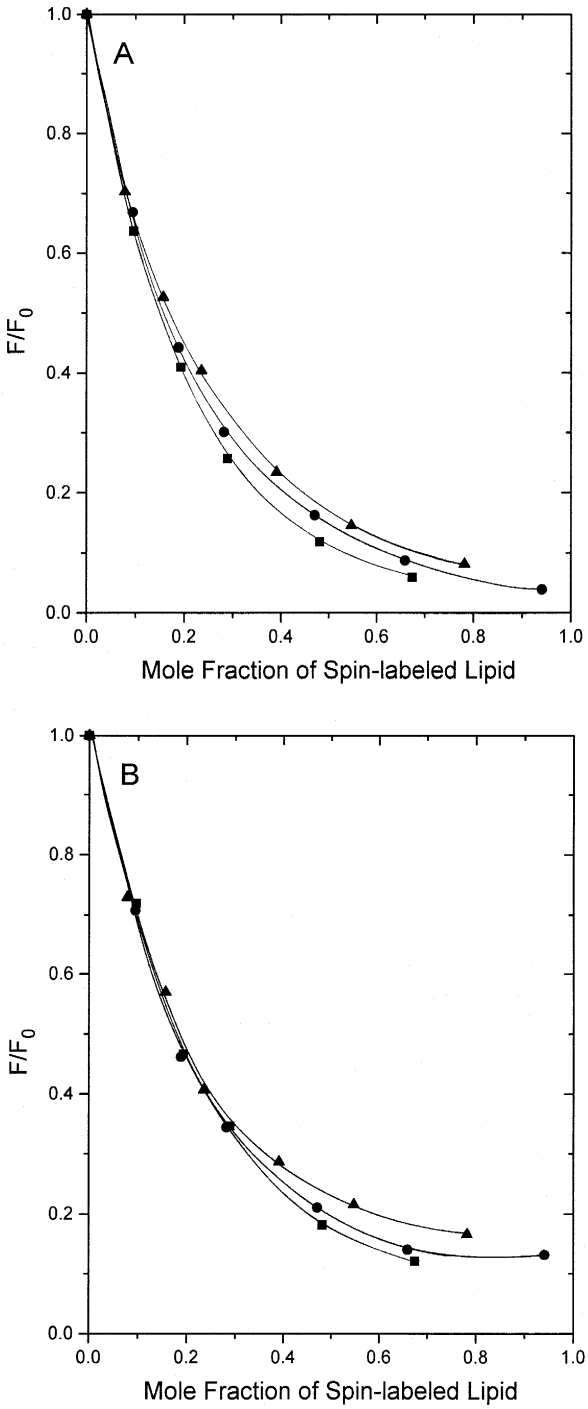

Fig. 1. Fluorescence in the presence of spin-labeled lipid, F, divided by fluorescence in the absence of spin-labeled lipid, F_0, vs. the mole fraction of spin-labeled lipid. (Panel A) Fluorescence quenching of 16:0,11-dansyl PC in multilamellar vesicles of DOPC and 5-SLPC (■), 10-SLPC (●), or 12-SLPC (▲). (Panel B) Fluorescence quenching of 18:1,4-dansyl PC in multilamellar vesicles of DOPC and 5-SLPC (■), 10-SLPC (●), or 12-SLPC (▲).

Table 1
Depth of dansyl labels

Fluorescent molecule	Spin-label pair used for quenching analysis	Calculated distance of fluor from bilayer center, z_{cF} (Å)	R_c (Å) [a]
16:0,11-Dansyl PC	5-12	10.1	
	5-10	11.1	
	10-12	8.8	
	All pairs	10.0 ± 1.0 [b]	10.3 ± 0.2
18:1,4-Dansyl PC	5-12	9.2	
	5-12	9.8	
	10-12	8.5	
	All pairs	9.1 ± 0.9	9.5 ± 0.4
Di 16:0 NBD-PE	5-12	11.9 ± 0.5	
	5-12 [c]	11.4	
16:0,12-NBD PC	5-12	9.9 ± 0.9	
	5-12 [c]	10.5	

[a] Calculated from Eq. (1) using data obtained with 5-SLPC and 12-SLPC.
[b] The numbers after the ± sign represent standard deviation.
[c] These values of z_{cf} were determined by Abrams and London [10], using the 5-SLPC/12-SLPC pair. Like our values, they may differ from the true fluor-to-bilayer-center distances (see text).

of the fluor attached to the four carbon acyl chain shows that the fluor was apparently located 9.1 Å from the bilayer center (Table 1). The locations of the fluors in 16:0 NBD-PE and 16:0,12-NBD PC were analyzed for comparison to the dansyl fluors. We obtained apparent distances from bilayer center of 11.9 and 9.9 Å, respectively, for these probes. As shown in Table 1, these distances are in excellent agreement with those obtained by Abrams and London [10], using the same 5-SLPC/12-SLPC quencher pair. However, using a Tempo-PC/5-SLPC pair, Abrams and London [10] determined that di 16:0 NBD-PE and 16:0,12-NBD PC were 18.9 and 19.8 Å from the bilayer center, respectively.

Fluorescence emission maxima for the 16:0,11-dansyl PC, the 18:1,4-dansyl PE, and the dansyl-PE fluorophores are shown in Fig. 2. The emission maxima of all three fluors in DOPC vesicles were higher or similar to those of the fluors in methanol, suggesting that both 16:0,11-dansyl PC and 18:1,4-dansyl PC, as well as dansyl-PE, existed in polar environments within the bilayer. Wagonner and Stryer [2] determined that dansyl-PE in PC vesicles had an emission maximum similar to dansyl-PE in methanol.

Fluorescence polarization of the two fluorescent PCs in immiscible mixtures of DPPC and DLiPC is shown in Fig. 3. Panel A shows the steady-state polarization ratios of 16:0,11-dansyl PC and Panel B shows the polarization ratios of 18:1,4-dansyl PC as a function of the amount of solid phase present. In both cases, the polarization values were quite low; however, the polarization of 16:0,11-dansyl PC was lower

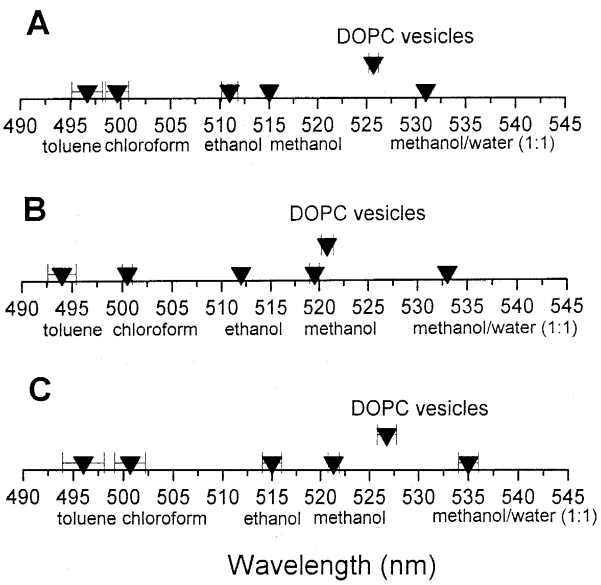

Fig. 2. Fluorescence emission maxima of 16:0,11-dansyl PC (A), dansyl-PE (B), and 18:1,4-dansyl PC (C) in solvents and in DOPC vesicles.

than that of 18:1,4-dansyl PC. This suggests that the environment of the dansyl group attached to the 11-carbon acyl chain allowed more motional freedom than that of the dansyl group attached to the 4-carbon acyl chain.

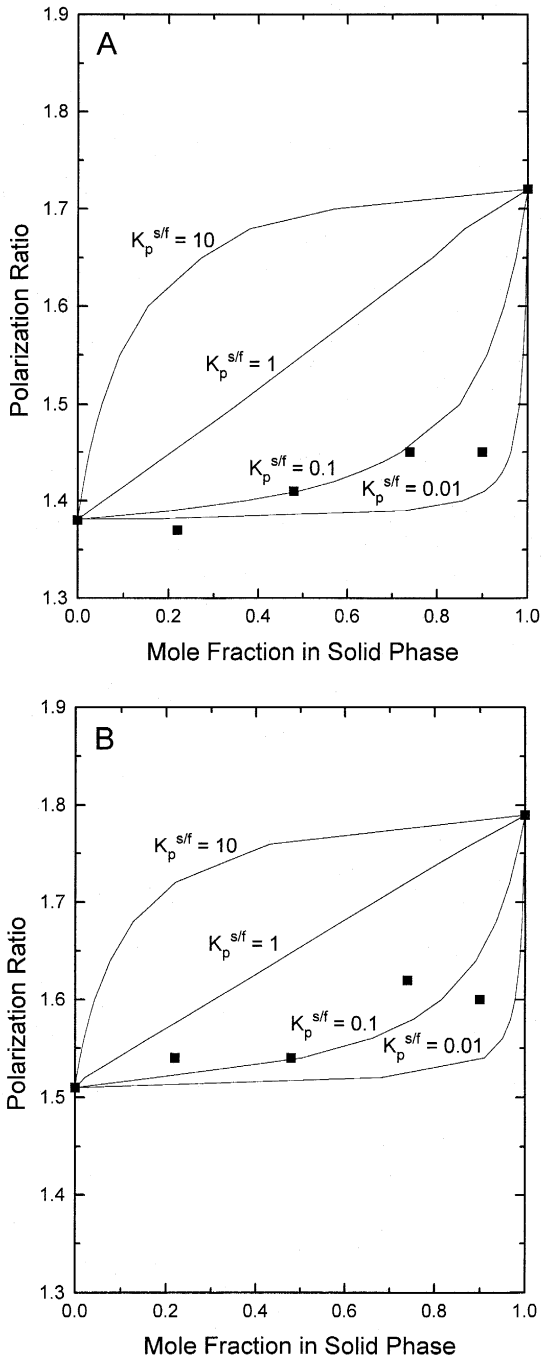

Fig. 3. Fluorescence polarization ratio of dansyl PCs vs. the mole fraction of solid-phase lipid in DPPC/DLiPC mixtures at 5°C. (Panel A) 16:0,11-dansyl PC; (Panel B) 18:1,4-dansyl PC. The smooth curves were calculated from Eq. (3) for the $K_p^{s/f}$ values indicated.

Table 2
Fluid–solid partition coefficients of dansyl PCs

Fluorescent molecule	$K_p^{s/f}$
16:0,11-Dansyl PC	0.08 ± 0.03
18:1,4-Dansyl PC	0.18 ± 0.14

As also can be seen from Fig. 3, the polarization ratio of the solid/fluid lipid mixtures, even when solid lipid accounted for 90% of the total lipid, remained low. This indicates that both dansyl PCs partitioned strongly into fluid phase lipid, with neither fluorescent lipid accomodated well in solid phase PC. The solid–fluid partition coefficients were calculated from Eq. (3) and are shown in Table 2.

4. Discussion

Previously, several phospholipids with polar fluors attached to acyl chains have been found to be located near the bilayer surface. For example, 16:0,6-NBD PC and 16:0,12-NBD PC have been localized to the polar/hydrocarbon interface region [6,10].

Parallax analysis of the location of 16:0,11-dansyl PC and 18:1,4-dansyl PC demonstrated that these fluors occupied similar positions in the bilayer. The analysis made it clear that the fluor of 16:0,11-dansyl PC occupied a shallower position in DOPC bilayers than would be expected if the 11-carbon acyl to which the fluor was attached was extended into the bilayer. Abrams and London [10] showed that the actual depth of shallow fluors was often less than that determined by quenching with 5-SLPC and 12-SLPC. A more accurate determination of the depth of shallow fluors can be obtained by parallax analysis using 5-SLPC and Tempo-PC. A long-term lack of commercial availability of high quality doxyl-fatty acids and their phospholipids derivatives has prevented us from undertaking that analysis. Instead, we employed analysis of the emission spectra of the fluors to gain more information about the location of the dansyl fluors. Both 18:1,4-dansyl PC and 16:0,11-dansyl PC fluors were found to occupy positions more polar than methanol. Wagonner and Stryer [2] interpreted an environment with the polarity of methanol as

being in the vicinity of the glycerol backbone of the phospholipids. Taken together, the data demonstrate the fluors of 16:0,11-dansyl PC and 18:1,4-dansyl PC exist at similar depths within the bilayer and that the location they occupy is very polar in character, and thus must be near or at the hydrocarbon/polar interface.

Because the fluor of 16:0,11-dansyl PC was localized to a polar environment at approximately the same depth as the fluor of 18:1,4-dansyl PC, it seems clear that the 11-carbon acyl chain, to which the dansyl group was attached, must have been bent back toward the bilayer surface. The bending created a conformation for the fluorescent phosphatidylcholine that was not easily accommodated in a solid-phase bilayer, as demonstrated by the low solid–fluid lipid partition coefficient determined by steady-state fluorescence polarization measurements. On the other hand, the data suggest that fluor of 18:1,4-dansyl PC occupied a position allowing less motional freedom than the fluor of 16:0,11-dansyl PC. The data tended to show that this probe had a higher fluid–solid partition coefficient than 16:0,11-dansyl PC, even though the unsaturated fatty acid in the 1-position would have directed the probe toward fluid-phase lipid [11]. A higher partition coefficient is consistent with the idea that the fatty acyl group of 18:1,4-dansyl PC was extended, since an extended chain would be more easily accommodated into solid-phase lipid than the bent fatty acyl chain of 16:0,11-dansyl PC.

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