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Alterations in membrane dynamics elicited by amphiphilic compounds are augmented in plasmenylcholine bilayers

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The dynamics of binary mixtures of choline glycerophospholipids and lysophospholipids were examined by fluorescence spectroscopy to compare and contrast the effects of each subclass of lysophospholipids on plasmenylcholine and phosphatidylcholine membrane motional characteristics. The decrease in steady-state anisotropy resulting from the introduction of lysoplasmenylcholine into plasmenylcholine bilayers was 4–6-fold greater than that manifest from the introduction of lysophosphatidylcholine into phosphatidylcholine bilayers (i.e., $\Delta r = 0.017$ vs. 0.004 or 0.011 vs. 0.002 at 5 C° and 10 C° above their phase transition temperatures, respectively). Lysoplasmenylcholine was also more potent than lysophosphatidylcholine in perturbing the dynamics of membrane bilayers comprised of phosphatidylcholine as measured by alterations in the steady-state anisotropy of the diphenylhexatriene probe. Finally, lipid matrices comprised of plasmenylcholine were uniformly more susceptible to amphiphilic perturbation (mediated by lysoplasmenylcholine, lysophosphatidylcholine or long chain acylcarnitine) than matrices comprised of phosphatidylcholine. Collectively, these results demonstrate that accumulation of plasmalogen catabolites resulting from activation of plasmalogen-selective phospholipases A_2 can potentiate alterations in membrane dynamics during signal transduction in plasmalogen-enriched bilayers.

Introduction

Stimulation of mammalian cells is accompanied by the activation of multiple phospholipases which collectively contribute to the propagation of biologic information. Alterations in membrane dynamics elicited by the products of phospholipase catalysis (e.g., lysophospholipids) modulate the kinetics of critical transmembrane proteins, perturb the affinity of cellular receptors for their ligands and modify the frequency and type of interactions between protein complexes participating in signal transduction (see Refs. 1–5). Accordingly, substantial efforts have focused on delineating the importance of lysophospholipids as biochemical modulators of diverse physiologic and pathophysiologic processes.

Recent studies have demonstrated both the predominance of plasmalogen molecular species in specific subcellular membrane compartments [6,7] and the existence of plasmalogen selective phospholipases A_2 in several tissues including myocardium [8,9], platelets [10], lung [11] and smooth muscle [12]. Although it has been implicitly assumed that alterations in membrane dynamics elicited by phospholipase A_2 catalyzed hydrolysis of plasmalogen-enriched membranes are similar to those previously demonstrated in phosphatidylcholine bilayers, the effects of amphiphilic compounds on plasmenylcholine membrane dynamics are unknown. Since plasmenylcholine and phosphatidylcholine bilayers have separate and distinct membrane dynamics [13] and conformational motifs [14,15] and since lysoplasmenylcholine has a substantially different molecular geometry than lysophosphatidylcholine, we compared alterations in membrane dynamics induced

Abbreviations: PhosCho, 1,2-dihexadecanoyl-*sn*-glycero-3-phosphocholine (dipalmitoylphosphatidylcholine); LPhosCho, 1-hexadecanoyl-*sn*-glycero-3-phosphocholine (lysophosphatidylcholine); PCarn, palmitoylcarnitine; DPH, 1,6-diphenyl-1,3,5-hexatriene; 3-AS, 3-(9-anthroyloxy)stearic acid; 16-AP, 16-(9-anthroyloxy)palmitic acid; LPlasCho, 1-*O*-(*Z*)-hexadec-1′-enyl-*sn*-glycero-3-phosphocholine (lysoplasmenylcholine); PlasCho, 1-*O*-(*Z*)-hexadec-1′-enyl-2-hexadecanoyl-*sn*-glycero-3-phosphocholine (plasmenylcholine); POPOP, *p*-bis[2-(5-phenyloxazolyl)]benzene.

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by lysoplasmenylcholine to those induced by lysophosphatidylcholine in both plasmenylcholine and phosphatidylcholine vesicles utilizing fluorescence spectroscopy. We now report that lysoplasmenylcholine is substantially more potent in altering membrane dynamics than lysophosphatidylcholine and that plasmenylcholine bilayers are more sensitive to perturbations by amphiphilic constituents than phosphatidylcholine bilayers. Collectively, these results underscore the potential importance of subclass specific alterations in membrane dynamics during signal transduction in membrane domains enriched in plasmalogen molecular species.

Materials and Methods

Materials

1,2-Dihexadecanoyl-*sn*-glycero-3-phosphocholine (dipalmitoylphosphatidylcholine (PhosCho)) and 1-hexadecanoyl-*sn*-glycero-3-phosphocholine (lysophosphatidylcholine (LPhosCho)) were obtained from Avanti Polar Lipids (Alabaster, AL). Palmitoylcarnitine (PCarn) was purchased from Sigma Chemical Company (St. Louis, MO). All fluorescent probes (1,6-diphenyl-1,3,5-hexatriene (DPH), 3-(9-anthroyloxy)stearic acid (3-AS) and 16-(9-anthroyloxy)palmitic acid (16-AP)) were obtained from Molecular Probes, Inc. (Eugene, OR) and were checked for purity by TLC prior to utilization. Palmitoyl chloride was purchased from Nu Check Prep, Inc. (Elysian, MN). *N,N*-Dimethyl-4-aminopyridine was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). HPLC solvents were obtained from Baxter Healthcare Corp. (Muskegon, MI). Other chemicals and reagents were obtained from Sigma Chemical Company (St. Louis, MO).

Preparation of lysoplasmenylcholine and plasmenylcholine. Homogeneous 1-*O*-(*Z*)-hexadec-1′-enyl-*sn*-glycero-3-phosphocholine (lysoplasmenylcholine (LPlasCho)) was prepared by modifications of previously described methods [16,17]. Briefly, bovine heart choline glycerophospholipids were subjected to alkaline methanolysis, extracted by the Bligh and Dyer method [18], and purified by preparative HPLC utilizing tandem Dynamax Macro-HPLC silica columns (each 21.5 mm × 25 cm; Rainin Instrument Co., Woburn, MA) employing a gradient of chloroform/methanol. Individual molecular species of LPlasCho were further resolved by preparative reverse phase HPLC utilizing an Econosil octadecyl silica column (10 mm × 25 cm; 10 μm particles (Alltech Associates, Inc. Deerfield, IL)) in conjuction with a mobile phase comprised of methanol/water/acetonitrile (57:23:20, v/v/v) containing 20 mM choline chloride. 1-*O*-(*Z*)-Hexadec-1′-enyl-2-hexadecanoyl-*sn*-glycero-3-phosphocholine (PlasCho) was prepared utilizing LPlasCho, palmitoyl chloride and large amounts (2 mole equivalents) of *N,N*-dimethyl-4-aminopyridine [19,20]. The resultant PlasCho was purified utilizing tandem Dynamax Macro-HPLC silica columns (each 21.5 mm × 25 cm) employing a linear gradient of chloroform/methanol (PlasCho eluted at 70:30 chloroform/methanol). Residual *N,N*-dimethyl-4-aminopyridine and a small percentage of the plasmenylcholine isomer (*sn*-2 phosphodiester resulting from isomerization) were subsequently removed by rechromatography utilizing the same stationary phase with a linear gradient of hexane/isopropanol/water from 48.5:48.5:3 to 46:46:8 (v/v/v).

Preparation of multilamellar and unilamellar vesicles. Choline glycerophospholipids were dissolved in chloroform in the absence or presence of selected mole fractions of amphiphiles (i.e., LPhosCho, LPlasCho or PCarn) at a constant total lipid concentration. To the dissolved mixtures, an aliquot of a freshly prepared solution of fluorescent probe (e.g., DPH, 3-AS or 16-AP), previously dissolved in chloroform/methanol (2:1, v/v), was subsequently added to yield a molar ratio of lipid/probe of 500:1. Organic solvents were evaporated to dryness utilizing N_2 prior to exhaustive evacuation at 50 mTorr for at least 2 h. The lipid mixtures were resuspended by addition of 3.5 ml of buffer (10 mM Tris-HCl, pH 7.5 at 25°C) to yield a final solution containing 0.4 mM total lipid. Multilamellar PlasCho and PhosCho vesicles were prepared by heating suspensions above the phase transition temperature (50°C) for 2 min prior to vigorous vortexing for 1 min in the presence of glass beads (all operations were performed under N_2). Heating and vortexing cycles were repeated five times and all dispersions were maintained above the phase transition temperature prior to utilization. Unilamellar vesicles (for fluorescence lifetime and differential tangent experiments) were prepared by sonicating these suspensions with a Vibra Cell Model VC600 sonicator (Sonics & Materials, Inc., Danbury, CT) utilizing a power level of 2 at a duty cycle of 50% for 10 min at 50°C under a N_2 atmosphere.

Fluorescence spectroscopy of plasmenylcholine and phosphatidylcholine vesicles. All fluorescence measurements were made utilizing a SLM 4800C spectrofluorometer (SLM Instruments, Urbana, IL) equipped with an acousto-optic Debye-Sears light modulator (operating at 6, 18 and 30 MHz) and two monochromators. Fluorescence polarization was performed utilizing a T-format with three Glan-Thompson polarizers. Temperature throughout samples was maintained by continuous stirring in a jacketed cuvette holder with an isothermal bath. Temperature was monitored with a thermistor (model YSI421, VWR Scientific) and found to be accurate within ±0.1°C.

Measurements of steady-state anisotropies were performed essentially as described by Lakowicz [21].

When DPH was utilized as a probe, excitation wavelength was 360 nm (2-nm slit width) and emission wavelength was 430 nm monitored through a 418 nm cutoff filter (KV418; Schott, Duryea, PA). Experiments utilizing the probes 3-AS and 16-AP employed 364 nm excitation (2-nm slit width) and 450 nm emission (utilizing a 418 nm cutoff filter). Fluorescence lifetimes were measured by the phase-modulation method (a modulation frequency of 18 MHz was employed) utilizing POPOP as the external lifetime reference solution [22]. Differential tangent polarized phase fluorometry was performed as described previously [23–25]. Phase differences of DPH in plasmenylcholine or phosphatidylcholine vesicles were measured at a modulation frequency of 18 MHz. After selected fluorescence measurements of either plasmenylcholine or phosphatidylcholine, the purity of the sample was checked by thin-layer chromatography utilizing two different solvent systems [13,19] which each demonstrated a single intense spot after iodine staining.

Membrane mobility calculations

Since DPH behaves as a hindered isotropic rotator in lipid bilayers [25–28] a modified Perrin-Weber equation was utilized to characterize the molecular dynamics of phospholipid membranes with the DPH probe [28,29]

$$r = r_\infty + (r_0 - r)/6R\tau \quad (1)$$

where R is the rotational rate (radians/s) of the fluorophore, τ is the fluorescence lifetime, r_0 is the steady-state fluorescence anisotropy in the absence of depolarizing rotations and r_∞ is the limiting anisotropy of the fluorophore. For this work the value of 0.390 for r_0 [27,28] was utilized. Differential polarized phase fluorometry (for measurement of tan Δ) was performed and results were analyzed utilizing the hindered isotropic rotator model of Weber [29]. In this formalism, R and r_∞ can be obtained through the measurements of r, tan Δ, and τ from solution of the following equation [28] (Eqn. 2) combined with Eqn. 1:

$$(m \tan \Delta)(2R\tau)^2 + (B \tan \Delta - 3A)(2R\tau) + (C \tan \Delta - A) = 0 \quad (2)$$

where

$$A = \omega\tau(r_0 - r)/3$$

$$B = (2r - 4r^2 + 2)/3$$

$$C = (m + m_0\omega^2\tau^2)/9$$

$$m = (1 + 2r)(1 - r)$$

The orientational order parameter (S_v) of fluorophore in phospholipid vesicles was calculated from the following equation [30,31]:

$$r_\infty = r_0 S_v^2 \quad (3)$$

The main phase transition temperatures (T_m) of vesicles were determined from the maximum of the first derivative of the temperature dependence of steady-state anisotropy. Alterations of the main phase transition temperature (ΔT_m) in vesicles produced by amphiphiles were quantified by determination of the magnitude of the shift in the maximum of first derivative profiles. The phase transition width (W_t) was obtained from the half-height width of the maximum peak in the first derivative profiles. The alteration of the phase transition width (ΔW_t) of vesicles (Table I) corresponds to the difference between the half-height widths.

TABLE I

Comparisons of the potency of amphiphiles to induce perturbations in the transition temperature (T_m) and transition width (W_t) of phosphatidylcholine or plasmenylcholine vesicles

Alterations of the phase transition temperature and width in phosphatidylcholine (PhosCho) or plasmenylcholine (PlasCho) oricles induced by 10 mol% of lysoplasmenylcholine (LPlasCho), lysophosphatidylcholine (LPhosCho) or palmitoylcarnitine (PCarn) were quantified by calculation of the decrease of the main phase transition temperature (ΔT_m) and the broadening of the phase transition (ΔW_t) from the measurements of the steady-state anisotropies of diphenylhexatriene (DPH), 3-(9-anthroyloxy)stearic acid (3-AS) or 16-(9-anthroyloxy)palmitic acid (16-AP) as described in Materials and Methods. Data represent the mean values ± S.E. from at least three independent sample preparations.

Probe	Vesicle matrix	No amphiphile		LPhosCho (10 mol%)		LPlasCho (10 mol%)		PCarn (10 mol%)	
		T_m	W_t	ΔT_m	ΔW_t	ΔT_m	ΔW_t	ΔT_m	ΔW_t
DPH	PhosCho	41.0±0.5	1.5±0.1	−0.7±0.1	0.6±0.2	−1.3±0.1	0.7±0.1	−0.7±0.1	0.6±0.2
	PlasCho	38.0±0.6	2.2±0.2	−1.1±0.1	0.9±0.1	−1.8±0.2	1.2±0.2	−1.0±0.1	0.9±0.1
3-AS	PhosCho	40.8±0.8	1.3±0.4	−0.6±0.2	0.9±0.4	−1.3±0.3	1.1±0.4	−0.7±0.2	0.9±0.4
	PlasCho	37.7±1.0	2.6±0.6	−1.0±0.3	1.2±0.7	−1.6±0.4	1.9±0.8	−1.0±0.3	1.4±0.8
16-AP	PhosCho	41.0±0.7	1.1±0.2	−0.6±0.1	0.8±0.3	−0.6±0.1	1.2±0.2	−0.6±0.1	0.8±0.2
	PlasCho	38.0±0.9	1.9±0.4	−0.8±0.1	1.2±0.4	−1.1±0.2	2.0±0.6	−0.8±0.2	1.3±0.4

Results

Comparisons of plasmenylcholine and phosphatidylcholine dynamics utilizing independent fluorescent probes

Initial experiments examined the temperature dependence of the steady-state fluorescence anisotropy of DPH (incorporated as a substitutional impurity) in PlasCho and PhosCho vesicles. Comparisons of the steady-state anisotropy of DPH in PlasCho and PhosCho vesicles demonstrated three substantive differences. First, the main transition temperature in PhosCho was 3 C° higher than that present in PlasCho (41° C vs. 38°C) (Fig. 1A; Table I). Second, the steady-state anisotropy of DPH in the liquid-crystal phase was significantly higher in PlasCho vesicles in comparison to PhosCho vesicles (Fig. 1A). Comparison of the steady-state anisotropy as a function of the relative temperature ($T - T_m$) demonstrate the substantive differences in the steady-state anisotropy of DPH in each phospholipid subclass (Fig. 1B). Third, although PhosCho vesicles contained an easily identifiable pretransition near 36°C (in agreement with previous studies [32,33]), measurable alterations in anisotropy during the pretransition in PlasCho vesicles were barely detectable (Fig. 1A).

Characterization of dynamics near the hydrophobic-hydrophilic interface in PlasCho and PhosCho vesicles was facilitated through utilization of the probe 3-AS. The fluorescence anisotropy of 3-AS in plasmenylcholine vesicles was substantially larger than that in phosphatidylcholine vesicles above the phase transition temperature (Fig. 2A). In contrast, differences in fluorescence anisotropy between plasmenylcholine and phosphatidylcholine vesicles were not present within the membrane interior as ascertained by the probe 16-AP (Fig. 2B). Characterization of the dynamics of these probes documented the lower phase transition temperature in PlasCho vesicles compared to PhosCho vesicles and the minimal perturbations in the steady-state fluorescence anisotropy produced during the pretransition in PlasCho vesicles.

The mobility of DPH in plasmenylcholine and phosphatidylcholine vesicles can be characterized by the correlation relaxation rate (R) and limiting anisotropy (r_∞) which were calculated from the steady-state anisotropy, fluorescence lifetime and phase differential tangent as described in Materials and Methods. Calculation of the limiting anisotropy and orientational order parameter of probe demonstrated a substantially higher value for plasmenylcholine vesicles compared to phosphatidylcholine vesicles in the liquid-crystal state especially when comparing alterations utilizing the traditionally accepted relative temperature (Figs. 3A and 3B). The average rotational rates for DPH in plasmenylcholine and phosphatidylcholine vesicles were similar above the phase transition.

Alterations in the steady-state anisotropy of fluorescent probes induced by amphiphiles in plasmenylcholine and phosphatidylcholine vesicles

Introduction of pathophysiologically relevant mole fractions of LPhosCho into PhosCho bilayers resulted in a modest decrease in the phase transition temperature of PhosCho without statistically significant alterations in its steady-state anisotropy (Fig. 4A). In contrast, incorporation of both 5 and 10 mol% LPhosCho

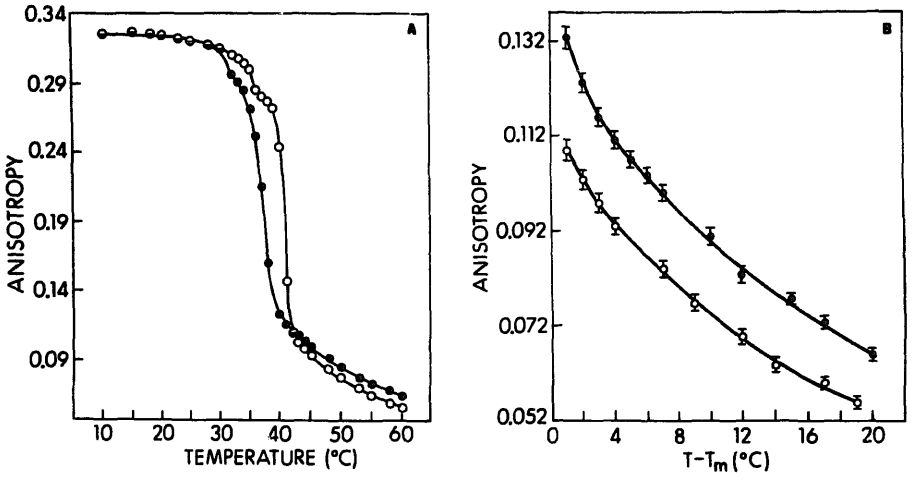

Fig. 1. Temperature dependence of the fluorescence anisotropy of diphenylhexatriene in phosphatidylcholine and plasmenylcholine vesicles. Vesicles comprised of phosphatidylcholine (○) or plasmenylcholine (●) containing diphenylhexatriene as a substitutional impurity (molar ratio 1:500) were prepared and the steady-state fluorescence anisotropy was quantified from 10 to 60°C (A) as described in Materials and Methods. To facilitate comparisons between these phospholipid subclasses direct comparisons of the fluorescence anisotropy as a function of relative temperature ($T - T_m$) were made from 1 to 20 C° (B). Data represent the mean value and standard error from at least five independent sample preparations. The main phase transition temperature (T_m) values of dipalmitoylphosphatidylcholine and dipalmitoylplasmenylcholine vesicles are 41 and 38°C, respectively, as determined in this study.

into bilayers comprised of PlasCho resulted in a broadening of the phase transition temperature and in statistically significant reductions (standard errors of data points are less than the height of the symbol) in the steady-state anisotropy of DPH above the phase transition (Fig. 4B). Incorporation of 5 or 10 mol% LPlasCho into vesicles comprised of PhosCho resulted in decreases in both the phase transition temperature and steady-state anisotropy of DPH in the liquid-crystal state (Fig. 5A). Importantly, incorporation of 5 or 10 mol% LPlasCho into vesicles comprised of PlasCho resulted in a dramatic decrease of the phase transition temperature accompanied by marked decreases in the steady-state anisotropy of DPH in the liquid-crystal phase (Fig. 5B, standard error of data points are less than the height of each symbol). Comparisons of the magnitude of the decrease in steady-state anisotropy in each of these binary systems clearly demonstrated the

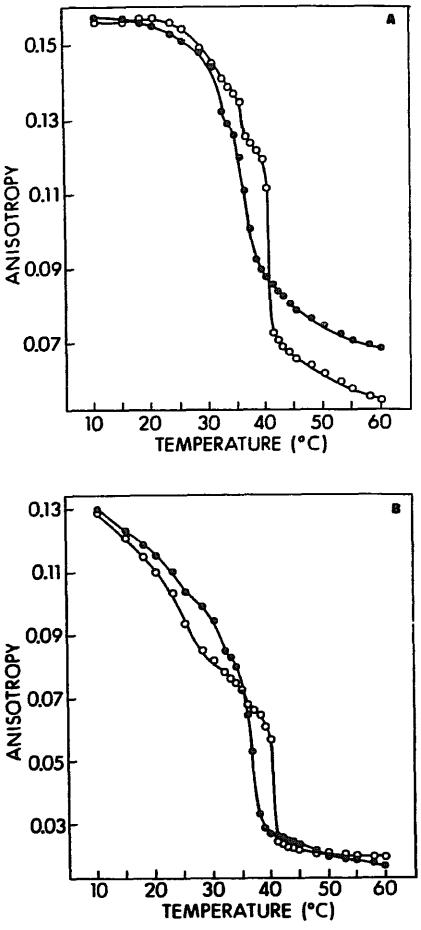

Fig. 2. Temperature dependence of the fluorescence anisotropy of 3-(9-anthroyloxy)stearic acid (3-AS) and 16-(9-anthroyloxy)palmitic acid (16-AP) in phosphatidylcholine and plasmenylcholine vesicles. Vesicles comprised of phosphatidylcholine (○) or plasmenylcholine (●) were prepared and the fluorescence anisotropy was quantified utilizing either 3-AS (A) or 16-AP (B) probe as a function of temperature as described in Materials and Methods. Data represent the mean values from at least five independent sample preparations and standard errors for data points are less than 5% of indicated value.

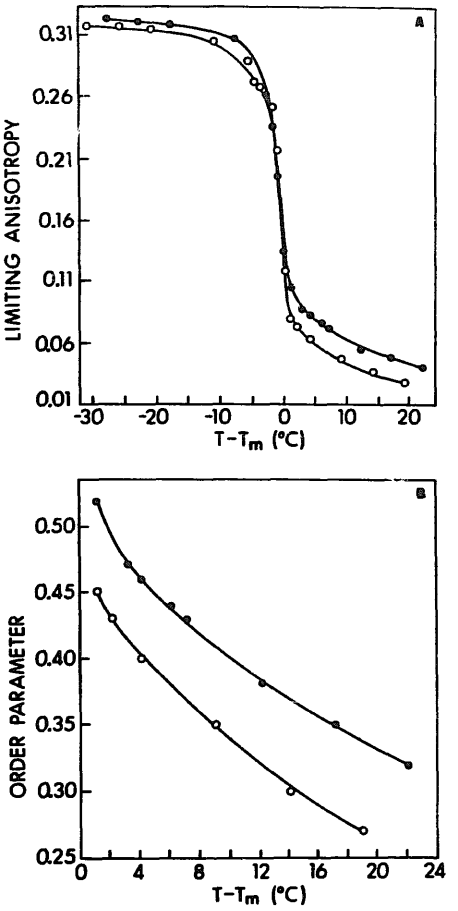

Fig. 3. Comparison of the limiting anisotropy and orientational order parameter of diphenylhexatriene in phosphatidylcholine and plasmenylcholine vesicles as a function of relative temperature ($T - T_m$). Vesicles comprised of phosphatidylcholine (○) or plasmenylcholine (●) containing diphenylhexatriene probe were prepared and fluorescence spectroscopy was performed as described in Materials and Methods. The limiting anisotropy (A) was calculated from the fluorescence lifetime, phase differential tangent and the steady-state anisotropy according to Eqns. 1 and 2. The orientational order parameter (B) was calculated from the limiting anisotropy according to Eqn. 3.

amplification of alterations elicited by the vinyl ether linkage when present in either the substitutional impurity or when present in the predominant matrix component (Fig. 6).

To determine if the observed alterations were due to *bona fide* alterations in rotational diffusion and molecular orientation or alternatively, reflected changes in DPH fluorescence lifetimes, the fluorescence lifetimes of these mixtures were determined by the phase modulation method as described in Materials and Methods for both phospholipid subclasses. Addition of 10 mol% of either subclass of lysophospholipid to either phosphatidylcholine or plasmenylcholine vesicles did not result in differences in the measured fluorescence lifetimes of DPH in these binary systems within experimental error. For example, the fluorescence lifetime of DPH in plasmenylcholine or

phosphatidylcholine vesicles at 50°C was 8.7 ± 0.4 ns ($\bar{X}$ ± SE) and addition of 10 mol% of either lysophospholipid subclass resulted in measured lifetimes of 8.7 to 8.9 ns.

Taken together, these results demonstrate that incorporation of lysophosphatidylcholine or lysoplasmenylcholine into plasmenylcholine bilayers has more potent effects on membrane dynamics than incorporation of these amphiphilic constituents into phosphatidylcholine bilayers. Similarly, the presence of a vinyl ether linkage in the lysophospholipid (e.g., lysoplasmenylcholine) increases its ability to perturb membrane dynamics in either phosphatidylcholine or plasmenylcholine vesicles in comparison to perturbations induced by lysophosphatidylcholine.

Since PCarn and LPhosCho have striking structural similarities (i.e., each has a quaternary amine, an ester linkage and similar regions of positive and negative

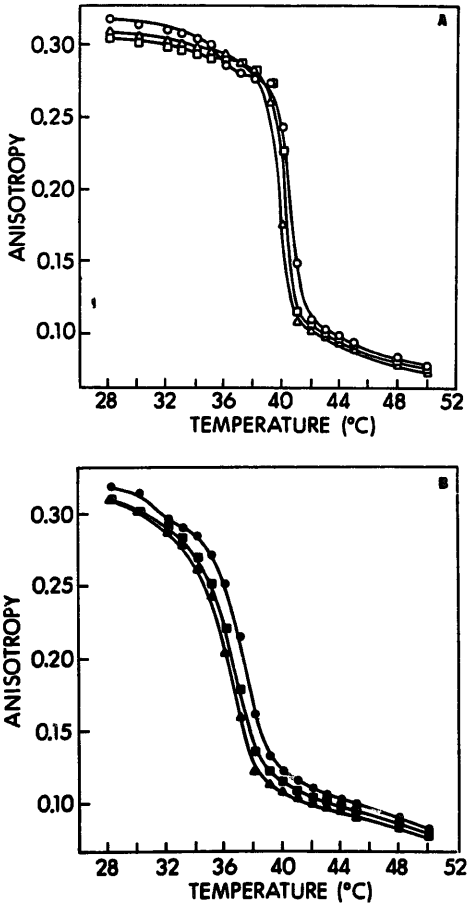

Fig. 4. Effects of lysophosphatidylcholine on the steady-state fluorescence anisotropy of diphenylhexatriene in phosphatidylcholine or plasmenylcholine vesicles. Vesicles comprised of phosphatidylcholine (A) or plasmenylcholine (B) containing 0 mol% (○, ●), 5 mol% (□, ■) or 10 mol% (△, ▲) lysophosphatidylcholine were prepared as described in Materials and Methods. Values of the steady-state anisotropy of diphenylhexatriene represent the mean value from at least three independent preparations. Standard errors are less than 5% of the indicated value (i.e., within the data point above the phase transition temperature).

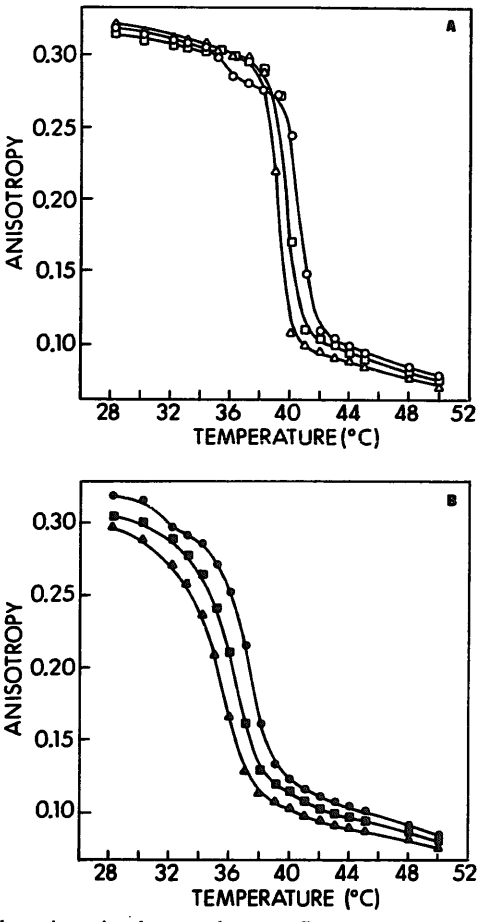

Fig. 5. Alterations in the steady-state fluorescence anisotropy of diphenylhexatriene induced by lysoplasmenylcholine in phosphatidylcholine or plasmenylcholine vesicles. Vesicles comprised of phosphatidylcholine (A) or plasmenylcholine (B) containing 0 mol% (○, ●), 5 mol% (□, ■) or 10 mol% (△, ▲) of lysoplasmenylcholine were prepared and the steady-state fluorescence anisotropy of diphenylhexatriene was determined as described in Materials and Methods. The results represent the mean value of at least three independent sample preparations and standard errors are within 5% of the reported value (i.e., within the data point above the phase transition temperature).

charge density [34]), additional experiments were performed to compare alterations in the steady-state anisotropy of DPH induced by PCarn in PlasCho and PhosCho vesicles. As anticipated, although only small changes in the phase transition temperature and steady-state fluorescence anisotropy were induced by pathophysiologically relevant concentrations of PCarn (0–10 mol%) in phosphatidylcholine bilayers (Fig. 7A), a broadening of the phase transition and a decrease in the steady-state anisotropy of DPH in the liquid-crystal phase was produced by PCarn in vesicles comprised of plasmenylcholine (Table I; Figs. 7A and 7B).

To specifically identify the effect of each of these three amphiphiles at specific locations in the membrane bilayer, comparisons of three fluorescent probes were performed. Each of the three amphiphiles tested (i.e., LPhosCho, LPlasCho and PCarn) decreased the

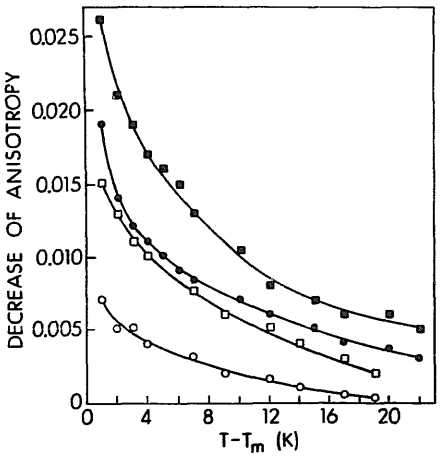

Fig. 6. Comparisons of the magnitude of lysophospholipid induced alterations in steady-state anisotropy of diphenylhexatriene in phosphatidylcholine and plasmenylcholine vesicles. Comparisons of the relative potency of lysophosphatidylcholine in phosphatidylcholine bilayers (○), lysoplasmenylcholine in phosphatidylcholine bilayers (●), lysophosphatidylcholine in plasmenylcholine bilayers (□) and lysoplasmenylcholine in plasmenylcholine bilayers (■) were performed by assessing the decrease in anisotropy elicited by incorporation of 10 mol% lysophospholipid into the phospholipid matrix as a function of relative temperature ($T - T_m$)

main phase transition temperature and increased the transition width in plasmenylcholine and phosphatidylcholine vesicles labeled with each fluorescent probe (i.e., 3-AS, 16-AP or DPH) (Table I). Furthermore, LPlasCho was markedly more potent than either LPhosCho or PCarn (which were similar) utilizing each probe. Finally, the potency of each amphiphile was greater in plasmenylcholine vesicles than in phosphatidylcholine vesicles utilizing 3-AS, 16-AP or DPH (Table I). Since similar effects were noted utilizing three separate probes with markedly different molecular geometries, these results demonstrate that the observed alterations reflect properties intrinsic to plasmenylcholine and phosphatidylcholine bilayers and do not reflect anomalous interactions of the probe with its phospholipid matrix.

Discussion

Since the conformation, dynamics and packing of plasmenylcholine and phosphatidylcholine are distinct [13–15,20,35], we anticipated that matrices comprised of each phospholipid subclass would differentially accommodate the incorporation of amphiphilic constituents. The present results demonstrate that liquid-crystal bilayers comprised of plasmenylcholine are more motionally restricted than those comprised of phosphatidylcholine which render them more susceptible to perturbation by the amphiphilic constituents that accumulate during cellular stimulation. Furthermore, assuming the tilt angle of the polar head group of both amphiphiles is similar, comparisons of molecular models of LPlasCho and LPhosCho demonstrate that the *cis* vinyl ether linkage in lysoplasmenylcholine results in dramatic alterations of the molecular geometry of LPlasCho compared to LPhosCho at the hydrophobic-hydrophilic interface (Fig. 8). Thus, the increased steric bulk of lysoplasmenylcholine renders it more potent than lysophosphatidylcholine as a modifier of membrane dynamics in both plasmenylcholine and phosphatidylcholine matrices. Finally, the largest perturbations in membrane dynamics in the four binary systems examined were produced by incorporation of lysoplasmenylcholine into plasmenylcholine bilayers.

Previous theories on the mechanism of lysophospholipid induced alterations in membrane packing have attributed the 'fluidization' of the membrane induced by lysophospholipids to packing defects resulting from the absence of the acyl constituent at the *sn*-2 position [36,37]. The differential propensity of plasmenylcholine

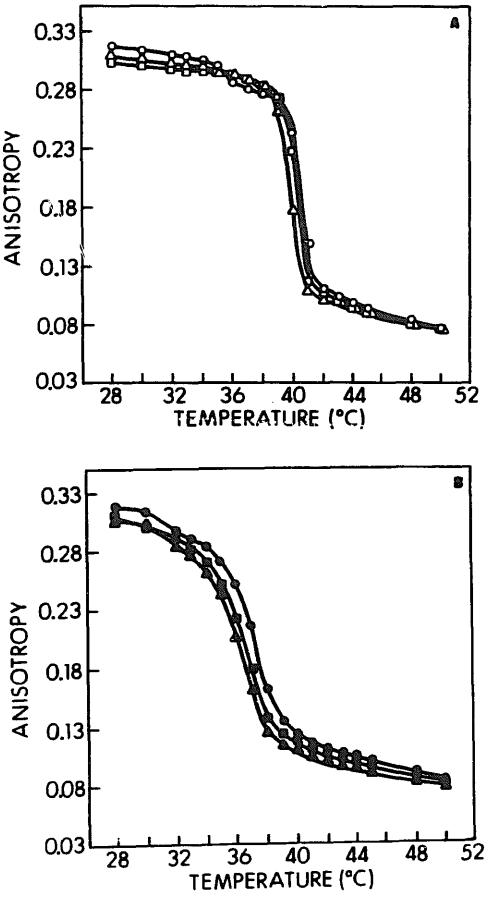

Fig. 7. Effects of palmitoylcarnitine on the steady-state fluorescence anisotropy of diphenylhexatriene in phosphatidylcholine or plasmenylcholine vesicles. Vesicles comprised of phosphatidylcholine (A) or plasmenylcholine (B) containing 0 mol% (○, ●), 5 mol% (□, ■) or 10 mol% (△, ▲) palmitoylcarnitine were prepared and the fluorescence anisotropy of diphenylhexatriene was measured as described in Materials and Methods. Values of the steady-state anisotropy represent the mean value from at least three independent preparations. Standard errors for data points are within 5% of the indicated value.

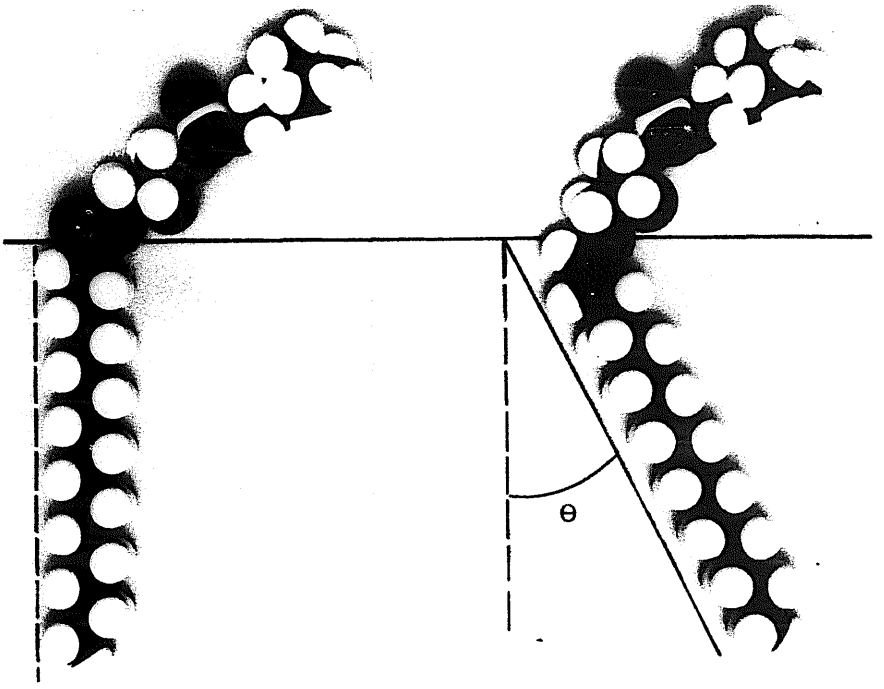

Fig. 8. Comparison of the molecular structures of lysophosphatidylcholine and lysoplasmenylcholine. Space filling models of lysophosphatidylcholine (left) and lysoplasmenylcholine (right) were constructed and superimposed onto a schematic of a membrane bilayer indicating the membrane surface (———) and the membrane director (— — —). Lysophosphatidylcholine was positioned on these axes by parallel alignment of its aliphatic chain with the membrane director in conjunction with the observed tilt angle for the polar head group in phosphatidylcholine initially quantified by Seelig et al. [39]. Lysoplasmenylcholine was aligned with the same relative tilt angle of the polar head group resulting in a substantial angle (θ) reflecting the relatively larger steric bulk of lysoplasmenylcholine in comparison to lysophosphatidylcholine.

and phosphatidylcholine bilayers to perturbations induced by lysophospholipids is likely attributable to the decreased cross-sectional molecular area and the resultant tighter packing in plasmenylcholine vesicles compared to phosphatidylcholine vesicles [14,35]. Accordingly, introduction of amphiphilic constituents into plasmenylcholine bilayers results in greater perturbations (i.e., packing defects) than does their introduction into more loosely packed phosphatidylcholine bilayers. Similarly, the increased steric bulk conferred by the *cis* double bond in the vinyl ether linkage in lysoplasmenylcholine compared to the ester linkage in lysophosphatidylcholine renders the lysophospholipid produced from phospholipase A_2 hydrolysis of plasmalogens more potent than its monoacyl counterpart in perturbing membrane dynamics. Collectively, these results suggest that the magnitude of alterations in membrane dynamics resulting from hydrolysis of plasmalogen enriched membranes are substantially larger than those previously anticipated from studies utilizing model membranes comprised of diacyl phospholipids and their metabolites.

Although many of the biochemical mechanisms leading to the activation of cells by augmentation of phospholipase activity are now clear (e.g., interaction of prostaglandins, leukotrienes and inositol trisphosphate with their cellular receptors), the molecular mechanisms which couple alterations in membrane dynamics to perturbations in membrane function are less well-defined. Previous consideration of the sequelae of lysophospholipid accumulation in activated cells has been confined to the effects of lysophosphatidylcholine and lysophosphatidylethanolamine on model membranes comprised of diacyl phospholipids. The recent demonstration of subcellular membranes predominantly comprised of plasmalogen molecular species [6,7] and the dramatic activation of plasmalogen selective phospholipases during cellular perturbation [38] in conjunction with the results of the present study suggest that previous perspectives be broadened to consider the amplification of alterations in membrane dynamics during signal transduction which result from hydrolysis of cellular membranes predominantly comprised of plasmalogen molecular species.

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