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Dynamics of binary mixtures of plasmenylcholine/arachidonic acid and phosphatidylcholine/arachidonic acid – a study using fluorescence and NMR spectroscopy

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Arachidonic acid is selectively released during signal transduction in many cell types. To examine the effects of physiologically relevant amounts of arachidonic acid on membrane bilayers, alterations in membrane dynamics induced by arachidonic acid were investigated utilizing fluorescence and nuclear magnetic resonance spectroscopy. We demonstrate that decreases in the fluorescence steady-state anisotropy of diphenylhexatriene are induced by incorporation of physiologically relevant amounts (i.e., 5 mol%) of arachidonic acid into either phosphatidylcholine or plasmenylcholine membrane bilayers. Furthermore, examination of the motional dynamics of the bis-allylic protons in arachidonic acid by analyses of their spin-spin relaxation times demonstrated that these protons are more restrained when arachidonic acid is present as a substitutional impurity in plasmenylcholine vesicles than in phosphatidylcholine vesicles. Collectively, these results demonstrate that arachidonic acid, when present in physiologically relevant mole fractions, can modify the molecular dynamics of biological membranes and that the motional dynamics of arachidonic acid in membrane bilayers is influenced by the type of covalent linkage present in the proximal portion of the *sn*-1 aliphatic chain in host bilayer matrices.

Introduction

Although arachidonic acid is rapidly released during signal transduction by the activation of phospholipases A_2 [1,2] in many types of eukaryotic cells, the effects of non-esterified arachidonic acid on membrane dynamics have not been defined. In some cells (e.g., cardiac myocytes), accelerated hydrolysis of endogenous cellular phospholipids is highly selective for phospholipids containing arachidonic acid and is topographically localized to the plasma membrane compartment [3,4]. Thus, it is likely that the plasma membrane of activated cells accumulates, at least transiently, substantial amounts of arachidonic acid during cellular stimulation [5]. Recent studies have demonstrated that plasmenylcholine and phosphatidylcholine bilayers have substantially different membrane dynamics [6,7] and conformational motifs [8,9]. Since arachidonoylated plasmalogen molecular species are the predominant phospholipid constituents present in the plasma membrane of cardiac mytocytes [10,11] and plasmalogen selective phospholipases A_2 are activated during at least some types of cellular stimulation [12], we examined alterations in membrane dynamics elicited by arachidonic acid in vesicles comprised of plasmenylcholine or phosphatidylcholine utilizing fluorescence and nuclear magnetic resonance spectroscopy. We now report that physiologically relevant mole percentages of arachidonic acid alter the molecular dynamics of both plasmenylcholine and phosphatidylcholine bilayers and that the dynamics of arachidonic acid in plasmenylcholine and phosphatidylcholine bilayers are substantially different.

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Abbreviations: DMAP, *N,N'*-dimethyl-4-aminopyridine; DNS-PE, *N*-(5-dimethylaminonaphthalene-1-sulfonyl)dipalmitoyl-L-α-phosphatidylethanolamine; DPH, 1,6-diphenyl-1,3,5-hexatriene; LPlasCho, 1-*O*-(*Z*)-hexadec-1'-enyl-*sn*-glycero-3-phosphocholine; NBD-PE, *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)dipalmitoyl-L-α-phosphatidylethanolamine; PO PhosCho, 1-hexadecanoyl-2-octadec-9'-enoyl-*sn*-glycero-3-phosphocholine; PO PlasCho, 1-*O*-(*Z*)-hexadec-1'-enyl-2-octadec-9'-enoyl-*sn*-glycero-3-phosphocholine.

Materials and Methods

Materials

Oleic acid and arachidonic acid were purchased from Nu Chek Prep (Elysian, MN). 1-Hexadecanoyl-2-octadec-9′-enoyl-*sn*-glycero-3-phosphocholine (PO PhosCho) was obtained from Avanti Polar Lipids (Alabaster, AL). 1-*O*-(*Z*)-Hexadec-1′-enyl-2-octadec-9′-enoyl-*sn*-glycero-3-phosphocholine (PO PlasCho) was prepared as described previously [13]. The mass and purity of these compounds were determined by acid methanolysis and capillary gas–liquid chromatography [10,14]. The purity was also checked by thin-layer chromatography on silica LK6D plates (Whatman) with both acidic (chloroform/acetone/methanol/acetic acid/water, 6:8:2:2:1, by volume) and basic (chloroform/methanol/28% ammonium hydroxide, 65:25:5, by volume) mobile phases and subsequent iodine staining. [^{3}H]Dipalmitoylphosphatidylcholine and [^{14}C]inulin were supplied by NEN (Boston, MA). All fluorescent probes including 1,6-diphenyl-1,3,5-hexatriene (DPH), *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)dipalmitoyl-L-α-phosphatidylethanolamine (NBD-PE) and *N*-(5-dimethylaminonaphthalene-1-sulfonyl)dipalmitoyl-L-α-phosphatidylethanolamine (DNS-PE) were purchased from Molecular Probes (Eugene, OR) and were used without further purification. D_2O (99.96%) was obtained from Cambridge Isotope Laboratories (Woburn, MA). Other chemicals used in these experiments were of the highest purity commercially available and most were purchased from Sigma (St. Louis, MO).

Preparation of samples for fluorescence spectroscopy

Both choline glycerophospholipids (PO PlasCho or PO PhosCho) and individual probes (1:250 or 1:125 molar ratio of probe/lipid for anisotropy or lifetime studies, respectively) in the presence or absence of arachidonic acid were codissolved in $CHCl_3$/MeOH (2:1, v/v) in the desired molar ratio prior to evaporation of the solvent to dryness (initially utilizing a nitrogen stream with subsequent exhaustive evacuation at 10 mTorr for at least 2 h). Next, lipid mixtures were resuspended by sonication after addition of 3 ml of buffer (10 mM Tris-HCl, 1 mM EGTA, pH 7.5 at 24°C; previously degased by nitrogen bubbling for at least 10 min) to yield a final solution containing 0.4 mM total lipid. Small unilamellar vesicles were obtained by high energy sonication (3 min at a power level of 1.5 employing a 50% duty cycle) utilizing a Vibra Cell Model VC600 sonicator (Sonics Materials, Danbury, CT) under a nitrogen atmosphere.

Preparation of phospholipid vesicles for nuclear magnetic resonance spectroscopy

Appropriate amounts of homogeneous PO PlasCho or PO PhosCho with or without either 5 mol% arachidonic acid or cholesterol (30 mM final phospholipid concentration in the absence of cholesterol and 40 mM total lipid concentration in experiments containing cholesterol (molar ratio of phospholipid/cholesterol of 7:3)) were initially dried with a nitrogen stream prior to exhaustive evacuation at 10 mTorr for at least 4 h. Subsequently, 1 ml of 100 mM sodium phosphate in D_2O (pD = 7.5 at 25°C) was added, and the mixture was sonicated under a nitrogen atmosphere (5 min at a power level of 1.5 employing a 30% duty cycle from a Vibra Cell Model VC600 sonicator followed by cooling on ice). Next, an additional 5 min sonication at room temperature under a nitrogen atmosphere was performed as above. The sonicate was centrifuged at $30\,000 \times g_{max}$ for 10 min to remove small amounts of titanium particles and multilamellar vesicles. The final lipid/arachidonic acid molar ratio was nearly equal to that of the initial mixture as determined by capillary gas chromatography after acid methanolysis of lipid vesicles [10,14].

Determination of surface area to volume ratios in phospholipid subclasses

To determine the surface area to volume ratio of different choline glycerophospholipid subclasses, the ratio of lipid and trapped volume of unilamellar vesicles was calculated. First, unilamellar vesicles comprised of [^{3}H]dipalmitoylphosphatidylcholine were prepared in the presence of [^{14}C]inulin by sonication twice for 2.5 min (cooled on ice between sonication periods) at a power level of 1 employing a 30% duty cycle. (Vibra Cell Model VC600 sonicator, Sonics Materials). An aliquot (200 μl) was then loaded onto a Sephadex G-50 column packed in a 3 ml disposable syringe [15], and vesicles were eluted by centrifugation of the column at $200 \times g_{max}$ for 4 min. This procedure was repeated once more to further remove the last traces of unincorporated inulin. Next, radioactivity in ^{3}H and ^{14}C in aliquots of column eluents were quantified utilizing a Beckman LS-1701 liquid scintillation counter. The ratios of entrapped inulin to phospholipids were calculated and utilized for comparisons of the relative size of vesicles comprised of different lipids.

Fluorescence spectroscopy

All fluorescence measurements were made utilizing an SLM 4800C spectrofluorometer (SLM Instruments, Urbana, IL) equipped with an acousto-optic Debye-Sears light modulator and two monochromators. Measurements of fluorescence anisotropy were performed utilizing a T format with three Glan-Thompson polarizers. Temperatures during experiments were varied between 25°C and 45°C in increments of 5 C° and were monitored utilizing a thermistor (model YSI421, VWR Scientific) inserted into the sample chamber. The sample temperature was kept constant utilizing an isother-

mal bath and found to be accurate within ±0.1 C°. All samples were continuously stirred to minimize photobleaching during experiments. Measurements of steady-state anisotropies were performed essentially as described by Lakowicz [16], employing excitation wavelengths of 358 nm (4 nm slit width), 455 nm (4 nm slit width) and 342 nm (8 nm slit width) for DPH, NBD-PE, and DNS-PE probes, respectively. Emission wavelengths of 430 nm (16 nm slit width, through a 418 nm cutoff filter), 536 nm (8 nm slit width, through a 515 nm cutoff filter), and 525 nm (16 nm slit width, through a 470 nm cutoff filter) were utilized for each probe, respectively. Vesicles without the probes were also prepared to assure that the light scattering component of the fluorescence signal was low enough to be ignored utilizing these experimental settings. Phase-modulation lifetimes of NBD-PE and DNS-PE were taken using a modulation frequency of 6 MHz at 25°C as described previously [16].

Nuclear magnetic resonance spectroscopy

Proton NMR spectroscopy of phospholipid vesicles was performed utilizing a Varian XL-300 spectrometer operating at 300 MHz. The sample temperature during experiments was 37 ± 0.5°C. The Carr-Purcell-Meiboom-Gill T_2 method ($CPMGT_2$) [17] was utilized to determine ^1H spin-spin relaxation times. Spectra were collected after a series of spin-echo cycle times (D_2) ranging from 0.5 ms to 0.5 s separated by a delay time of 4.5 s (greater than $5T_1$ of all protons) [7]. Nine points were utilized for each T_2 determination and T_2 values of selected peaks were calculated by a least-squares best-fit routine. After the T_2 experiments, the sample was checked by thin-layer chromatography to verify that significant degradation of phospholipid constituents had not occurred during vesicle preparation and T_2 measurements.

Results

Arachidonic acid induced alterations in the steady-state anisotropies of fluorescence probes incorporated into vesicles comprised of plasmenylcholine or phosphatidylcholine

The influence of arachidonic acid on the motional characteristics of fluorescence probes located in either the membrane interior or at the hydrophobic/hydrophilic interface was assessed by quantifying alterations induced by arachidonic acid in the steady-state anisotropies of DPH, NBD-PE and DNS-PE in phosphatidylcholine and plasmenylcholine membrane bilayers. Since the type of motion sensed by the probe is determined by its location and orientation in the lipid bilayer, as well as its emission transition moment, initial experiments examined alterations in the fluorescence anisotropy of DPH incorporated into the membrane

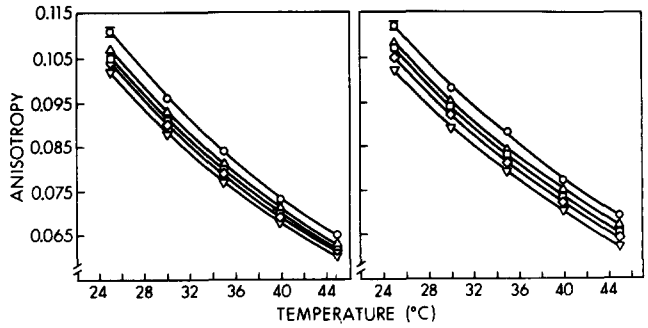

Fig. 1. Effects of arachidonic acid on the steady-state fluorescence anisotropy of diphenylhexatriene in phosphatidylcholine or plasmenylcholine vesicles. Vesicles comprised of phosphatidylcholine (left panel) or plasmenylcholine (right panel) containing 0 mol% (○), 5 mol% (△), 10 mol% (□), 15 mol% (◇) or 20 mol% (▽) arachidonic acid were prepared, and the steady-state fluorescence anisotropy of the diphenylhexatriene probe in these membranes was determined as described in Materials and Methods. Data represent the means ± S.D. of three independent preparations.

interior as a substitutional impurity (1:250) in binary mixtures comprised of phosphatidylcholine/arachidonic acid or plasmenylcholine/arachidonic acid. Incorporation of increasing amounts of arachidonic acid into either plasmenylcholine or phosphatidylcholine resulted in a decrease in the fluorescence anisotropy of the probe in each subclass of choline glycerophospholipids over the entire temperature range examined (25–45°C) (Fig. 1). For purposes of comparison, we also examined the effect of oleic acid on the molecular dynamics of plasmenylcholine and phosphatidylcholine vesicles through measurements of steady-state anisotropy of DPH incorporated into vesicles comprised of each subclass. The addition of oleic acid into plasmenylcholine or phosphatidylcholine vesicles resulted in qualitatively similar, albeit smaller magnitude, decreases in the fluorescence anisotropy of the DPH

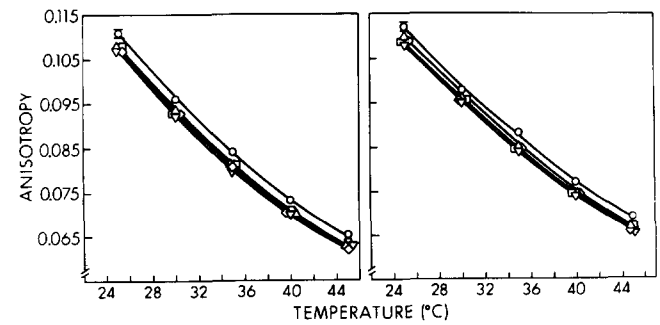

Fig. 2. Alterations in the steady-state fluorescence anisotropy of diphenylhexatriene induced by oleic acid in phosphatidylcholine or plasmenylcholine vesicles. Vesicles comprised of phosphatidylcholine (left panel) or plasmenylcholine (right panel) containing 0 mol% (○), 5 mol% (△), 10 mol% (□), 15 mol% (◇) or 20 mol% (▽) oleic acid were prepared, and the steady-state fluorescence anisotropy of the diphenylhexatriene probe in these membranes was determined as described in Materials and Methods. Data represent the means ± S.D. of three independent preparations.

probe in both plasmenylcholine and phosphatidylcholine vesicles (Fig. 2). Comparisons of Figs. 1 and 2 demonstrate that the decrease in anisotropy induced by the tetraunsaturated arachidonic acid was substantially larger than the decrease mediated by the monounsaturated oleic acid in vesicles of each subclass of choline glycerophospholipids.

To examine the effects of arachidonic acid on the motional characteristics of the polar headgroup, the fluorescence probes NBD-PE and DNS-PE were utilized. Comparisons of the fluorescence anisotropy of NBD-PE in plasmenylcholine to that in phosphatidylcholine vesicles demonstrated substantial differences in the motional characteristics of the two choline glycerophospholipid subclasses (e.g., $r = 0.145 \pm 0.001$ vs. 0.100 ± 0.001 for plasmenylcholine and phosphatidylcholine, respectively, at 25°C). The observed differences are not due to different lifetimes of the NBD-PE and DNS-PE probes in these two types of vesicles since there were no significant differences in the fluorescence lifetimes of these fluorophores in vesicles comprised of each lipid subclass (e.g., $\tau = 8.2 \pm 0.6$ ns vs. $\tau = 8.1 \pm 0.5$ ns (NBD-PE) and $\tau = 14.1 \pm 0.4$ ns vs. $\tau = 14.7 \pm 0.6$ ns (DNS-PE) for phosphatidylcholine and plasmenylcholine, respectively, at 25°C). Furthermore, these differences are not due to different radii of curvature in these two types of vesicles since they are almost the same size as determined by their similar surface area to volume ratios utilizing [^{14}C]inulin and ^{3}H-labeled phospholipids.

The addition of 5 mol% arachidonic acid induced no changes in the fluorescence anisotropy of the probe

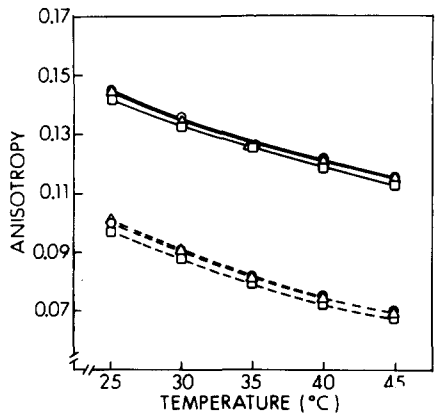

Fig. 3. Comparisons of the alterations induced by arachidonic acid in the steady-state anisotropy of *N*-(7-nitrobenz-2-oxa-1,3,-diazol-4-yl)dipalmitoyl-L-α-phosphatidylethanolamine (NBD-PE) in phosphatidylcholine and plasmenylcholine vesicles. Vesicles comprised of phosphatidylcholine (------) or plasmenylcholine (———) containing 0 mol% (○), 5 mol% (△) or 10 mol% (□) arachidonic acid were prepared, and the steady-state fluorescence anisotropy of NBD-PE was measured as described in Materials and Methods. Data represent the mean values of at least three independent preparations. The standard deviation is no larger than the height of the symbols.

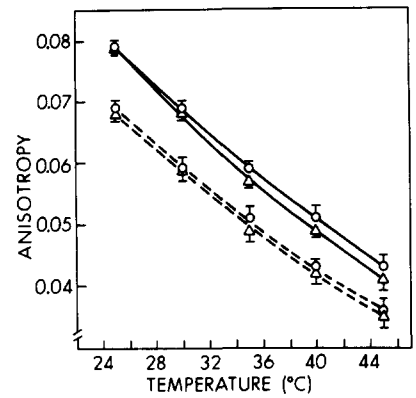

Fig. 4. Effects of arachidonic acid on the steady-state fluorescence anisotropy of *N*-(5-dimethylaminonaphthalene-1-sulfonyl)dipalmitoyl-L-α-phosphatidylethanolamine (DNS-PE) in phosphatidylcholine and plasmenylcholine vesicles. Vesicles comprised of phosphatidylcholine (------) or plasmenylcholine (———) containing 0 mol% (○) or 5 mol% (△) arachidonic acid were prepared, and the steady-state fluorescence anisotropy of DNS-PE was determined as described in Materials and Methods. Data represent the means ± S.D. of three independent preparations.

NBD-PE in vesicles comprised of either plasmenylcholine or phosphatidylcholine. Small decreases in anisotropy were observed in the above vesicles if arachidonic acid was further increased to 10 mol% with respect to phospholipids (Fig. 3).

Use of the DNS-PE probe also demonstrated large differences in the fluorescence anisotropy of the probe present in bilayers comprised of plasmenylcholine or phosphatidylcholine (e.g., $r = 0.079 \pm 0.001$ vs. 0.069 ± 0.001 for plasmenylcholine and phosphatidylcholine, respectively, at 25°C). However, almost no differences in the anisotropy of DNS-PE were induced by 5 mol% arachidonic acid in vesicles comprised of each phospholipid subclass (Fig. 4).

Spin-spin relaxation time (T_2) measurements of protons in the polar headgroup of phosphatidylcholine and plasmenylcholine and quantification of alterations induced by arachidonic acid

^{1}H-NMR spectra of phosphatidylcholine and plasmenylcholine vesicles demonstrated the anticipated resonances whose assignments were confirmed by homonuclear 2-dimensional correlation spectroscopy [13]. Since the CH_2-N and the N-CH_3 resonances were well resolved, the motional characteristics of the polar headgroup were quantified by assessing the transverse relaxation times of these protons by the Carr-Purcell-Meiboom-Gill method [17]. The T_2 values of the CH_2-N and the N-CH_3 group in phosphatidylcholine vesicles were significantly higher than those present in plasmenylcholine vesicles (Fig. 5). The addition of 5 mol% arachidonic acid resulted in modest increases in the T_2 values of the CH_2-N and N-CH_3 resonances present in phosphatidylcholine while arachidonic acid did not in-

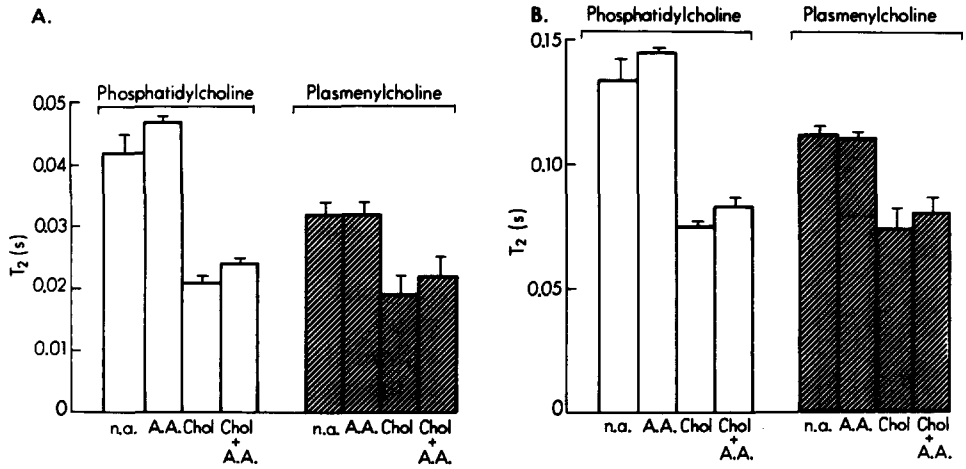

Fig. 5. Comparisons of the spin-spin relaxation times (T_2) of phosphatidylcholine and plasmenylcholine vesicles. Vesicles comprised of phosphatidylcholine or plasmenylcholine in the absence (n.a.) or presence of either 5 mol% arachidonic acid (A.A.) or cholesterol (molar ratio of phospholipid/cholesterol of 7:3) (Chol) or both cholesterol and arachidonic acid (Chol + A.A.) were prepared, and the T_2 values of the CH_2-N (A) and the N-CH_3 (B) protons were determined as described in Materials and Methods. Data represent the means ± S.D. of three independent preparations.

duce changes in the spin-spin relaxation times of these protons in plasmenylcholine vesicles (Fig. 5). The addition of cholesterol to phosphatidylcholine or plasmenylcholine vesicles resulted in substantial decreases in the spin-spin relaxation times of the CH_2-N and N-CH_3 resonances in each phospholipid subclass. Although there were significant differences in the T_2 values of these polar headgroup protons in each phospholipid subclass in the absence of cholesterol, the T_2 values of the CH_2-N and N-CH_3 resonances were nearly identical in binary mixtures containing either subclass in the presence of cholesterol. Incorporation of 5 mol% arachidonic acid resulted in modest increases in the T_2 values of both the CH_2-N and N-CH_3 protons in plasmenylcholine or phosphatidylcholine vesicles in the presence of 30 mol% cholesterol.

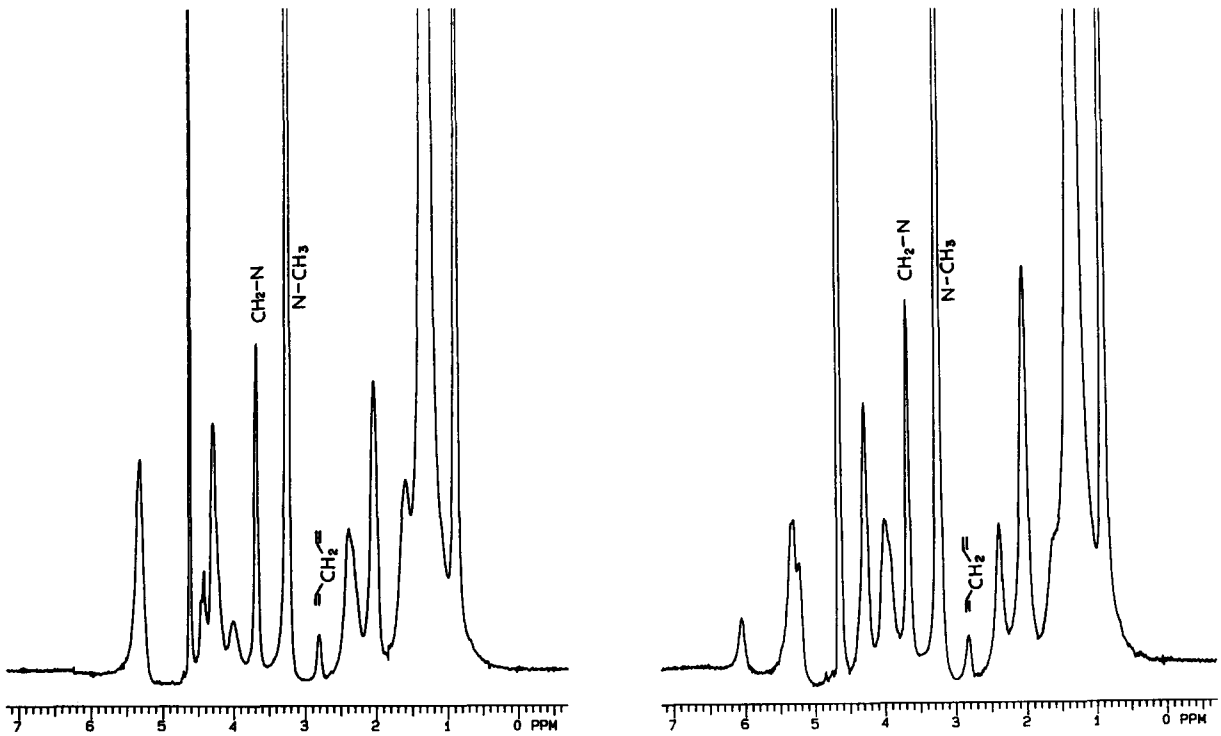

Fig. 6. Conventional 300 MHz ^{1}H-NMR spectrum of phosphatidylcholine/arachidonic acid or plasmenylcholine/arachidonic acid binary mixtures. Vesicles comprised of PO PhosCho (left panel) or PO PlasCho (right panel) containing 5 mol% arachidonic acid were prepared as described in Materials and Methods. Proton spectra were recorded at 37.0 ± 0.5°C at 300 MHz with the internal solvent peak (HOD at 4.63 ppm) as reference. The resonances corresponding to the CH_2-N, N-CH_3 and bis-allylic protons are indicated.

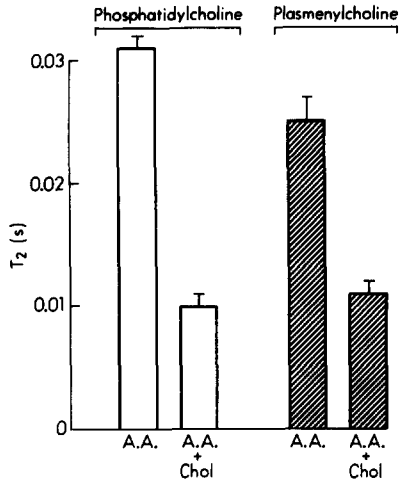

Fig. 7. Comparisons of the spin-spin relaxation times (T_2) of the bis-allylic protons in binary mixtures of phosphatidylcholine/arachidonic acid or plasmenylcholine/arachidonic acid and alterations induced by cholesterol. Vesicles comprised of phosphatidylcholine or plasmenylcholine containing 5 mol% A.A. in the absence (A.A.) or presence of cholesterol (molar ratio of phospholipid/cholesterol of 7:3) (A.A. + Chol) were prepared and measurements of the T_2 values of the bis-allylic protons were performed as described in Materials and Methods. Data represent the means ± S.D. of three independent preparations.

Comparison of the motional characteristics of arachidonic acid in phosphatidylcholine and plasmenylcholine vesicles in the presence or absence of cholesterol

^{1}H-NMR spectra of plasmenylcholine or phosphatidylcholine vesicles containing 5 mol% arachidonic acid demonstrated well resolved resonances at 2.8 ppm corresponding to the bis-allylic protons present in arachidonic acid (Fig. 6). To compare the motional characteristics of the central portion of the non-esterified arachidonic acid molecule incorporated into vesicles of each phospholipid subclass, spin-spin relaxation time measurements of the bis-allylic protons in each system were performed in the presence or absence of cholesterol. The motion of the bis-allylic protons of arachidonic acid in phosphatidylcholine vesicles was significantly more rapid than that manifested in arachidonic acid incorporated into plasmenylcholine vesicles (Fig. 7). Addition of cholesterol to either plasmenylcholine or phosphatidylcholine vesicles resulted in a decrease in the velocity of molecular motion of the bis-allylic protons in arachidonic acid incorporated into bilayers comprised of each phospholipid subclass. Although the motional characteristics of the bis-allylic protons in arachidonic acid were different in each phospholipid subclass in the absence of cholesterol, addition of cholesterol ablated the observed differences in the velocity of the motion of the bis-allylic protons of arachidonic acid in bilayers comprised of each choline glycerophospholipid subclass (Fig. 7).

Discussion

The results of the present study demonstrate that: (1) physiologically relevant amounts of arachidonic acid (i.e., ~ 5 mol%) [5] can modify the dynamics of plasmenylcholine and phosphatidylcholine membrane bilayers; and (2) the dynamics of the bis-allylic protons in arachidonic acid are substantially different in plasmenylcholine as compared to phosphatidylcholine bilayers.

While DPH is localized within the hydrophobic core of the membrane, it is distributed in a wide range along the bilayer normal [18]. Previous EPR investigation with 5-nitroxide stearic acid has shown that the disordering effect of unsaturated fatty acids is greater than that of saturated ones [19]. In agreement with the previous study [19], significant changes in molecular motion were found to be caused in dynamically different plasmenylcholine and phosphatidylcholine membranes by the presence of arachidonic acid. The effects of oleic acid on the motional characteristics of DPH in the interior of the bilayer were substantially smaller than those induced by arachidonic acid; these results suggest that increased motional freedom in the interior of the membrane reflects, at least in part, the number of spatial defects induced in the aliphatic chain lattice structure in mammalian membranes by olefinic linkages present in the non-esterified fatty acid.

Large differences in the fluorescence anisotropies of both NBD-PE and DNS-PE were present in phosphatidylcholine vesicles as compared to plasmenylcholine vesicles in the absence of arachidonic acid, which is consistent with the spin-spin relaxation measurements of the headgroup protons shown in Fig. 5. The observed differences are not due to the different radii of curvature in these two types of vesicles since they have similar surface area to volume ratios utilizing [^{14}C]inulin and ^{3}H-labeled phospholipids. Neither can the differences be attributed to different lifetimes of the NBD-PE and DNS-PE probes in these two types of vesicles. The incorporation of physiologically relevant amounts of arachidonic acid into phosphatidylcholine or plasmenylcholine host bilayers resulted in modest alterations in the fluorescence anisotropies of NBD-PE and DNS-PE in both subclasses. Prior results [20,21], as well as results obtained in this study, demonstrate that although the chromophores in both NBD-PE and DNS-PE are located near the hydrophobic/hydrophilic interface, they have markedly different fluorescence anisotropies (e.g., $r = 0.100 \pm 0.001$ for NBD-PE and $r = 0.069 \pm 0.001$ for DNS-PE in phosphatidylcholine membranes at 25.0°C).This is likely due to the combined influences of the different fluorescence lifetimes of each reporter group and their disparate conformation and dynamics at the membrane interface [22,23]. Thus, each probe interacts in a distinct fashion with

chemical entities present in the membrane interface and each undergoes different amounts of motional flux prior to photon emission.

The spin-spin relaxation times of protons in the polar headgroup in phosphatidylcholine and plasmenylcholine were remarkable for their substantially different transverse relaxation times for both the CH_2-N and the N-CH_3 protons in each phospholipid subclass. This observation that headgroups in phosphatidylcholine vesicles are less restrained than those in plasmenylcholine vesicles correlates well with data obtained using the NBD-PE and DNS-PE fluorescent probes and is reasonable due to the relatively upright orientation of the polar region in plasmenylcholine and the known tighter packing of plasmenylcholine in membrane bilayers [8]. The addition of small amounts of arachidonic acid had only modest effects on the transverse relaxation times in phosphatidylcholine vesicles and caused no demonstrable alterations in plasmenylcholine vesicles. The effects of arachidonic acid on the transverse relaxation times were also studied in vesicles containing cholesterol since large amounts of cholesterol is present in the plasma membranes (about 30–50 mol%), and it is believed to be a primary modulator of the properties of bilayers [24]. Although some prior studies utilizing ^{13}C- and ^{2}H-NMR have demonstrated only modest effects of cholesterol on the polar headgroup motion in multilamellar vesicles [25,26], the present results demonstrate that large changes in the motional regime of the polar headgroup in small unilamellar vesicles are induced by cholesterol as ascertained from proton spin-spin relaxation times. The differences likely arise from the different vesicle sizes and the different rates and types of mobility predominantly monitored in measurements of T_2 (both fast and slow motions in our studies). The effects of cholesterol on the measured T_2 values were large in phosphatidylcholine vesicles in comparison to plasmenylcholine vesicles, resulting in nearly equal T_2 values for each subclass in the presence of physiologically relevant amounts of cholesterol. These results may be attributable to the slower motion of the polar headgroup in plasmenylcholine (as a consequence of tighter packing) and the possibility that introduction of cholesterol into the membrane bilayer results in similar amounts of hydrogen binding of the 3′-OH in cholesterol with the phosphate moiety in both glycerophospholipid subclasses [27]. The addition of arachidonic acid in vesicles containing cholesterol caused no demonstrable effects on the measured T_2 values in both lipid subclasses.

Examination of the transverse relaxation times of the bis-allylic protons in arachidonic acid allows comparisons of both fast and slow motional regimes in the interior of the membrane in each choline glycerophospholipid subclass. The molecular motion of the bis-allylic protons of arachidonic acid was more rapid in phosphatidylcholine vesicles than in plasmenylcholine vesicles. These differences in the rate of molecular motion in phosphatidylcholine vesicles as compared to plasmenylcholine vesicles parallel those anticipated from prior EPR studies demonstrating that the motional dynamics of the nitroxide probe were substantially more rapid in phosphatidylcholine than in plasmenylcholine vesicles [6]. The addition of physiologically relevant amounts of cholesterol (i.e., ~30 mol%) resulted in a dramatic decrease in the transverse relaxation times of the bis-allylic protons of arachidonic acid incorporated into both plasmenylcholine and phosphatidylcholine vesicles, as well as changes in the T_2 values of protons in the polar headgroup region of both choline glycerophospholipid subclasses. It is interesting to note that cholesterol induces these alterations in the motional dynamics of vinyl ether containing phospholipids which are unable to form the 'typical' hydrogen bonds between the 3′-OH group of cholesterol and the *sn*-1 carbonyl present in diacyl phospholipids. Thus, in agreement with prior studies [6,28], the presence of the *sn*-1 ester linkage is not an obligatory requirement for interaction of mammalian phospholipids with cholesterol. Taken together, the present study demonstrates that the dynamics of both subclasses of choline glycerophospholipids are subject to modification by physiologically relevant amounts of arachidonic acid and that the dynamics of the arachidonic acid released during signal transduction are quite different in membranes enriched in phospholipids containing a vinyl ether linkage as compared to an ester linkage at the *sn*-1 position.

Acknowledgement

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