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Proton nuclear magnetic resonance studies on the molecular dynamics of plasmenylcholine/cholesterol and phosphatidylcholine/cholesterol bilayers

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Physiologically relevant molecular species of plasmenylcholine and phosphatidylcholine were synthesized and their molecular dynamics and interactions with cholesterol were compared by determination of salient proton spin-lattice relaxation times and apparent activation energies for ^{1}H-NMR observable motion. The molecular dynamics of PA PhosCho (1-hexadecanoyl-2-eicosatetra-5′,8′,11′,14′-enoyl-*sn*-glycero-3-phosphocholine) were substantially different from PA PlasCho (1-*O*-(*Z*)-hexadec-1′-enyl-2-eicosatetra-5′,8′,11′,14′-enoyl-*sn*-glycero-3-phosphocholine) in multiple regions of the bilayer. Furthermore, the fluidity gradient of PA PhosCho was larger than that of PA PlasCho as ascertained by ^{1}H spin-lattice relaxation time measurements. Introduction of cholesterol into each bilayer resulted in disparate effects on the dynamics of each subclass including: (1) increased motional freedom in the polar head group of PA PlasCho without substantial alterations in the dynamics of the polar head group of PA PhosCho; and (2) increased immobilization of the membrane interior in PA PlasCho in comparison to PA PhosCho. Analysis of Arrhenius plots of T_1 relaxation times demonstrated that the apparent activation energies for vinyl and bisallylic methylene proton NMR observable motion in PA PhosCho were greater than that in PA PlasCho. Thus, comparisons of spin-lattice relaxation times and apparent activation energies demonstrate that vesicles comprised of PA PlasCho and PA PhosCho possess differential molecular dynamics and distinct interactions with cholesterol. Collectively, these results underscore the significance of the conjoint presence of the vinyl ether linkage and arachidonic acid as an important determinant of membrane dynamics in specialized mammalian membranes.

Introduction

During the last decade the importance of membrane molecular dynamics as a primary determinant of the kinetic characteristics of transmembrane enzymes has become increasingly appreciated. Although many insights into the biologic sequelae of alterations in membrane conformation, dynamics and surface charge have been made, the large majority of previous studies have utilized model systems comprised of diacyl phospholipids containing saturated aliphatic chains at the *sn*-1 and *sn*-2 positions of the glycerol backbone. However, some subcellular membranes (e.g., myocardial sarcolemma [1] and sarcoplasmic reticulum [2]) contain a predominance of plasmalogen molecular species with arachidonic acid at the *sn*-2 position resulting in alterations in their molecular conformation, organization and dynamics. Since the sarcolemmal membrane of cardiac myocytes and other cells are highly enriched in both cholesterol [3–5] as well as arachidonylated plasmalogen molecular species [1,2,6,7], the dynamic interactions of these two lipids are of particular interest. No information is currently available on either the motional characteristics of arachidonylated plasmalogens or on their interactions with cholesterol.

Abbreviations: PO PhosCho, 1-hexadecanoyl-2-octadec-9′-enoyl-*sn*-glycero-3-phosphocholine; PA PhosCho, 1-hexadecanoyl-2-eicosatetra-5′,8′,11′,14′-enoyl-*sn*-glycero-3-phosphocholine; DMAP, *N*,*N*-dimethyl-4-aminopyridine; HPLC, high-performance liquid chromatography; LPlasCho, 1-*O*-(*Z*)-hexadec-1′-enyl-*sn*-glycero-3-phosphocholine (lysoplasmenylcholine); PO PlasCho, 1-*O*-(*Z*)-hexadec-1′-enyl-2-octadec-9′-enoyl-*sn*-glycero-3-phosphocholine; PA PlasCho, 1-*O*-(*Z*)-hexadec-1′-enyl-2-eicosatetra-5′,8′,11′,14′-enoyl-*sn*-glycero-3-phosphocholine; NMR, nuclear magnetic resonance.

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Although ^1H nuclear magnetic resonance spectroscopy utilizes naturally abundant nuclei and ^1H is the most sensitive naturally occurring nucleus, it has not been extensively utilized in studies of membrane dynamics. In large part, this has resulted from difficulties in relating spectral features to motional characteristics. However, experimentally determined proton spin-lattice relaxation times of sonicated vesicles are directly related to the rotational correlation time of each functional group at temperatures well above the main phase transition (e.g., physiologic temperature for arachidonylated plasmenylcholine) utilizing high observational magnetic frequencies [8]. Furthermore, the thermotropic dependence of T_1 values can be utilized to determine the activation energies for NMR observable motion to gain additional insight into salient differences in the dynamics of phospholipid subclasses and their modulation by cholesterol. In this report, we show that the effects of cholesterol on the proton spin-lattice relaxation times in plasmalogens and diacyl phospholipids are different both in the polar head group region and in the membrane interior and demonstrate that the activation energies which characterize the dynamics of the arachidonic acid chain in each phospholipid subclass are distinct.

Materials and Methods

Materials. 1-Hexadecanoyl-2-octadec-9′-enoyl-*sn*-glycero-3-phosphocholine (PO PhosCho) and 1-hexadecanoyl-2-eicosatetra-5′,8′,11′,14′-enoyl-*sn*-glycero-3-phosphocholine (PA PhosCho) were purchased from Avanti Polar Lipids, Inc. (Birmingham, AL) and found to be 98% pure. Cholesterol (Sigma Chemical Company, St. Louis, MO) was recrystallized from ethanol and dried under high vacuum for more than two hours prior to use. Oleoyl chloride and arachidonoyl chloride (sealed in individual ampules) were obtained from Nu Chek Prep, Inc. (Elysian, MN). *N,N*-Dimethyl-4-aminopyridine (DMAP) was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). All solvents were analytical reagent grade or HPLC grade.

Preparation of plasmenylcholine. Homogeneous lysoplasmenylcholine was prepared by modifications of previously described methods [9,10]. Briefly, bovine heart choline glycerophospholipids were subjected to alkaline methanolysis, the resultant lysophospholipids were extracted by the Bligh and Dyer method [11] and were purified by HPLC utilizing tandem Dynamax Macro silica columns (each 21.4 × 250 mm; Rainin Instrument Co., Woburn, MA) by application of a 1 L linear gradient of $CHCl_3$ to MeOH at a flow rate of 9–10 ml/min. Lysoplasmenylcholine eluted at 30% $CHCl_3$/70% MeOH (detected by thin-layer chromatography and iodine staining) with typical yields of 70%. Homogeneous 1-*O*-(*Z*)-hexadec-1′-enyl-*sn*-glycero-3-phosphocholine (LPlasCho) was obtained by isocratic reverse-phase HPLC utilizing an Econosil octadecyl silica column (10 × 250 mm; 10 micron particles (Alltech Associates, Inc., Deerfield, IL)) as previously described [12]. Homogeneous 1-*O*-(*Z*)-hexadec-1′-enyl-2-octadec-9′-enoyl-*sn*-glycero-3-phosphocholine (PO PlasCho) and 1-*O*-(*Z*)-hexadec-1′-enyl-2-eicosatetra-5′,8′,11′,14′-enoyl-*sn*-glycero-3-phosphocholine (PA PlasCho) were prepared utilizing LPlasCho, either oleoyl chloride or arachidonoyl chloride and DMAP as previously described [13]. The resultant plasmenylcholines were purified utilizing tandem Dynamax Macro-HPLC silica columns (each 21.4 × 250 mm) employing a linear gradient of chloroform/methanol (plasmenylcholine eluted at 70 : 30 chloroform/methanol by volume). Residual DMAP and the *sn*-2 phosphodiester isomer 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). Purified plasmenylcholines were characterized utilizing fast atom bombardment mass spectroscopy and proton NMR [13] as well as by demonstration of a single intense spot after TLC in two different solvent systems after iodine staining [14]. Purity of these plasmenylcholines was found to be greater than 95% by acid methanolysis and capillary gas-liquid chromatography (the 3–5% impurity resulted from C_{15} or C_{17} vinyl ether constituents at the *sn*-1 aliphatic chain [1,15]).

Preparation of NMR samples. Appropriate amounts of homogeneous phospholipids dissolved in chloroform were placed into a 5 ml conical vial prior to addition of recrystallized cholesterol (in chloroform). Organic solvent was evaporated to apparent dryness under a nitrogen stream prior to exhaustive evacuation at 50 mTorr for at least 4 h. The lipid mixtures were resuspended by addition of 0.7 mL of degassed buffer (0.1 M phosphate/D_2O buffer, pD 7.0/25°C). The suspension was sonicated utilizing twelve 30 s bursts from a Vibra Cell Model VC600 sonicator (Sonics Materials, Inc., Danbury, CT) at 40°C under a nitrogen atmosphere which resulted in the formation of small unilamellar vesicles. The sonicate was centrifuged at $30000 \times g_{max}$ for 20 min to remove small amounts of particulate matter. Exposure to light was minimized when utilizing polyunsaturated phospholipids. Alterations of the concentration of pure phospholipid vesicles from 20 mM to 60 mM or utilization of different sonication times or intensities had no discernible effects on the measurements of proton spin-lattice relaxation times after the suspension became optically transparent. Therefore, 40 mM of total lipid concentration was utilized in all experiments.

NMR spectroscopy. Proton NMR spectra of small unilamellar vesicles of lipids were obtained utilizing a Varian XL-300 spectrometer operating at 300 MHz (7.05 Tesla) equipped with a temperature controller

which regulated the sample temperature within 0.5 C°. Samples were equilibrated for at least 20 min at each observed temperature before data acquisition was begun. The fast inversion recovery method was utilized to determine spin-lattice relaxation times by the following pulse sequence [16]:

$$\underbrace{(90° - A_t\text{-D})}_{\text{not sampled}}\ \underbrace{(180° - \tau - 90° - A_t\text{-D})_n}_{\text{sampled}}$$

where $D' = A_t + D \leq 5T_1$. Then T_1 relaxation times can be obtained from:

$$\ln\left(\frac{I_\infty - I_\tau}{I_\infty}\right) = -\frac{\tau}{T_1} + \ln\left[2 - \frac{n-1}{n}\exp\left(-\frac{D'}{T_1}\right)\right] \quad (1)$$

by linear analysis of the left-hand term as a function of τ. In typical experiments in this study, $n = 40$, D varied from 3 to 6 s (depending on the experiment temperature) and at least eight values of τ for each T_1 measurement were made (the largest τ was over 10 times the longest T_1). In comparisons of the data obtained utilizing this method to those from the conventional inversion recovery method, no differences were observed in the range of experimental error. The maximum standard error was ±10% from at least three entirely independent sample preparations. After selected T_1 experiments, the sample purity was verified by thin-layer chromatography on silica LK60 plates (Whatman) utilizing two different solvent systems [14] which each demonstrated a single intense spot after visualization by iodine staining.

Results

Proton NMR spectroscopy of homogeneous mixed chain plasmenylcholines and phosphatidylcholines

^{1}H-NMR spectra of phosphatidylcholine and plasmenylcholine dissolved in $CDCl_3$ demonstrated the anticipated resonances whose assignments were confirmed by homonuclear two-dimensional correlation spectroscopy [13]. ^{1}H-NMR spectra of sonicated unilamellar vesicles comprised of binary mixtures of phospholipid (PA PhosCho or PA PlasCho)/cholesterol in phosphate buffer demonstrated the appearance of similar resonances, albeit with less resolution, due to the presence of complex dipolar interactions (Fig. 1). These spectra demonstrated that the choline methyl and methylene, aliphatic terminal methyl, vinyl, and bisallylic methylene proton resonances were sufficiently well resolved to investigate their molecular dynamics utilizing spin-lattice relaxation measurements. Similar spectra were obtained utilizing PO PhosCho and PO PlasCho except that the bisallylic proton resonances were not present and the vinyl proton resonances were less intense (spectra not shown).

Dynamics of vinyl and bisallylic methylene protons in the sn-2 position of arachidonylated plasmenylcholine and phosphatidylcholine vesicles

The molecular dynamics of the arachidonylated aliphatic chain in plasmenylcholine and phosphatidylcholine bilayers were determined by measurements of their corresponding T_1 relaxation times and apparent activation energies of the vinyl and bisallylic methylene protons in each subclass. The T_1 relaxation times of vinyl protons in PA PlasCho bilayers were smaller than those in PA PhosCho bilayers at each cholesterol concentration and temperature examined (Fig. 2A). As expected, the T_1 relaxation times of the vinyl protons decreased significantly with the addition of cholesterol in both PA PhosCho and PA PlasCho bilayers (e.g., decrease of 60% and 40% of T_1 relaxation times for PA PlasCho and PA PhosCho, respectively, after addition of 40 mol% cholesterol at all temperatures examined). The activation energies for vinyl proton motion in PA PlasCho bilayers were smaller than those present in PA PhosCho bilayers. The addition of cholesterol to PA PhosCho or PA PlasCho bilayers did not substantially alter the activation energies of the vinyl protons in either PA PhosCho or PA PlasCho bilayers (Table I).

The T_1 relaxation times of the bisallylic methylene protons in PA PhosCho and PA PlasCho bilayers con-

TABLE I

Apparent activation energies, E_a (kJ/mol), of NMR observable motions of phospholipid molecular functional groups in phospholipid/cholesterol bilayers obtained from Arrhenius plots of proton spin-lattice relaxation times

Functional group	Bilayer matrix	Mole fraction (%) of cholesterol in bilayer		
		0	20	40
ω CH_3	PA PhosCho	18.5	15.5	11.5
	PO PhosCho	18.1	14.0	9.1
	PA PlasCho	16.1	12.8	7.3
	PO PlasCho	19.0	9.1	6.7
-$(CH_2)_n$-	PA PhosCho	11.5	11.0	11.1
	PO PhosCho	12.7	12.1	11.2
	PA PlasCho	9.8	13.3	9.8
	PO PlasCho	14.5	10.6	9.8
=C-CH_2-C=	PA PhosCho	14.6	13.7	13.9
	PA PlasCho	11.8	12.3	12.0
-(CH=CH)$_4$-	PA PhosCho	15.5	16.6	15.8
	PA PlasCho	13.0	13.8	12.6
N-CH_2	PA PhosCho	14.9	13.9	14.1
	PO PhosCho	14.7	14.0	13.2
	PA PlasCho	13.0	14.7	14.3
	PO PlasCho	12.9	12.0	9.3
N-$(CH_3)_3$	PA PhosCho	18.1	17.1	16.8
	PO PhosCho	17.9	16.0	16.8
	PA PlasCho	15.1	17.2	15.8
	PO PlasCho	20.0	17.7	17.5

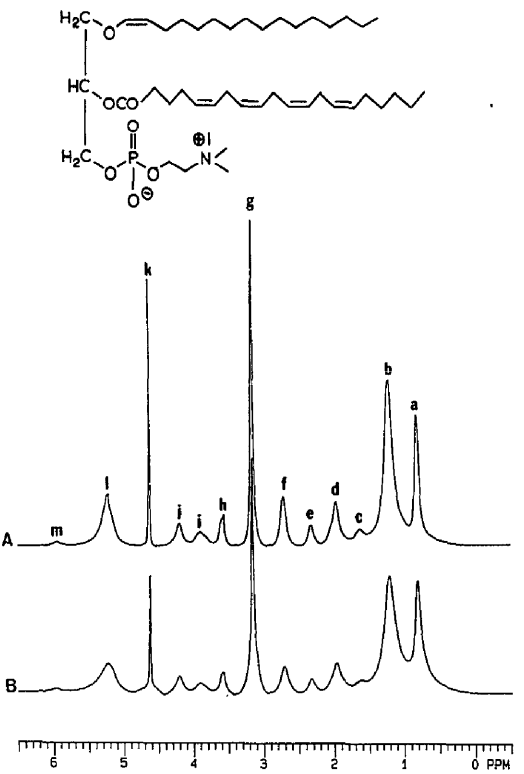

Fig. 1. 300 MHz ^{1}H-NMR spectra of sonicated unilamellar vesicles of 1-*O*-(*Z*)-hexadec-1′-enyl-2-eicosatetra-5′,8′,11′,14′,-enoyl-*sn*-glycero-3-phosphocholine (PA PlasCho, molecular formula as shown at top) containing 0 or 20 mol% cholesterol. Sonicated unilamellar vesicles (0.7 ml, 40 mM) comprised of PA PlasCho and 0 mol% (A) or 20 mol% (B) cholesterol were prepared and ^{1}H-NMR spectra were performed at 30°C utilizing a standard one-pulse sequence with 64 acquisitions and a data size of 16 K. Resonances were assigned after two-dimensional correlation spectroscopy as follows: a, aliphatic terminal methyl protons ($-CH_3$); b, aliphatic methylene protons ($-(CH_2)_n-$); c, β-methylene (C_3) protons in acyl chain; d, allylic methylene protons ($C{=}C-CH_2$); e, α-methylene (C_2) protons in acyl chain; f, bisallylic methylene protons ($C{=}C-CH_2-C{=}C$); g, choline methyl protons ($N-(CH_3)_3$); h, choline methylene protons ($N-CH_2$); i, *sn*-1,3 glycerol protons; j, $P-O-CH_2$ in choline and β-vinyl ether proton ($-O-C{=}CH-$); k, HOD; l, vinyl protons ($\Delta^{5,8,11,14}-CH{=}CH-$); m, α-vinyl ether proton ($O-CH{=}C-$).

taining 0–40 mol% cholesterol were shorter than those of vinyl protons in corresponding bilayers (compare Fig. 2A and Fig. 2B). Cholesterol altered the T_1 relaxation times of the bisallylic methylene protons in both PA PhosCho and PA PlasCho bilayers and the alterations were larger in PA PlasCho bilayers than in PA PhosCho bilayers (reduction of 16% for PA PlasCho vs. 10% for PA PhosCho by addition of 40 mol% cholesterol at all temperatures examined) (Fig. 2B). The activation energies of bisallylic protons in PA PlasCho bilayers were smaller than those present in PA PhosCho bilayers (Table I). Furthermore, the bisallylic methylene protons possessed lower activation energies for NMR observable motion than vinyl protons in both subclasses.

Dynamics of terminal methyl and aliphatic methylene protons in plasmenylcholine and phosphatidylcholine vesicles

To characterize the dynamics of *sn*-2 arachidonylated plasmenylcholine and phosphatidylcholine near the bilayer center, the T_1 relaxation times of the terminal methyl protons of the aliphatic chain were quantified. Terminal methyl protons in PA PhosCho bilayers had longer T_1 relaxation times at each temperature examined than corresponding protons in PA PlasCho bilayers (compare solid and dashed lines in Fig. 3A). Furthermore, the apparent spin-lattice relaxation times of the terminal methyl protons in each phospholipid subclass were reduced by addition of physiologically

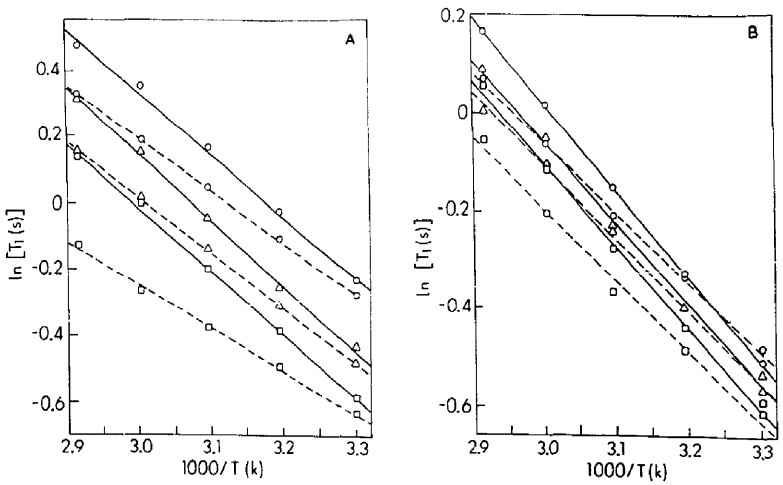

Fig. 2. Arrhenius plot of T_1 relaxation times of vinyl and bisallylic methylene protons in phosphatidylcholine and plasmenylcholine bilayers containing 0–40 mol% cholesterol. Sonicated unilamellar vesicles of PA PhosCho (———) or PA PlasCho (- - - - -) containing 0 (○), 20 (△) or 40 (□) mol% cholesterol were prepared and T_1 relaxation times of vinyl protons (A) and bisallylic methylene protons (B) were determined at 300 MHz utilizing the fast inversion recovery method as described in Materials and Methods. Data points are the mean value of at least three independent sample preparations. The standard errors are less than 10% of the mean value.

relevant amounts of cholesterol (0–40 mol% of membrane lipid). The cholesterol-induced reduction of T_1 relaxation times of terminal methyl protons in PA PlasCho bilayers was larger than that in PA PhosCho bilayers (Fig. 3A). It should be recognized that methyl protons in cholesterol contribute to the intensity of the terminal methyl resonance and its apparent spin-lattice relaxation time. To compare the effects of multiple *cis*

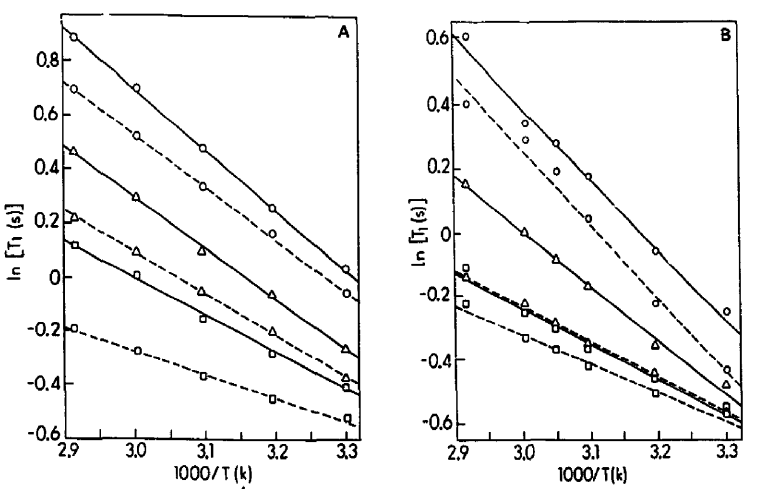

Fig. 3. Arrhenius plots of T_1 relaxation times of aliphatic terminal methyl protons in phosphatidylcholine and plasmenylcholine bilayers containing 0–40 mol% cholesterol. Sonicated unilamellar vesicles of PA PhosCho (———) or PA PlasCho (- - - - -) (A) and PO PhosCho (———) or PO PlasCho (- - - - -) (B) containing 0 (○), 20 (△) or 40 (□) mol% cholesterol were prepared and T_1 relaxation times of aliphatic terminal methyl protons were determined at 300 MHz utilizing the fast inversion recovery method as described in Materials and Methods. Data points are the mean value of at least three independent sample preparations. The standard errors are less than 10% of the mean value.

double bonds in the arachidonic acid chain to effects elicited by a single *cis* double bond, the T_1 relaxation times of the terminal methyl protons in the aliphatic chains of PO PhosCho and PO PlasCho bilayers were determined. The T_1 relaxation times of the terminal methyl protons in tetraenoic phospholipids (i.e., PA PhosCho and PA PlasCho) bilayers were substantially longer than those in monoenoic phospholipids (i.e., PO PhosCho and PO PlasCho). Furthermore, the T_1 relaxation times of the terminal methyl protons in PO PhosCho bilayers were significantly longer than those in PO PlasCho bilayers (compare solid and dashed lines in Fig. 3B); the T_1 relaxation times of the terminal methyl protons in both PO PhosCho and PO PlasCho bilayers were reduced by introduction of cholesterol (Fig. 3B); and the reduction of T_1 relaxation times of the terminal methyl protons in PO PlasCho bilayers by addition of cholesterol was much larger than that induced by cholesterol in PO PhosCho bilayers (Fig. 3B).

The apparent activation energies (E_a) of NMR observable motions of the terminal methyl protons in each phospholipid subclass were calculated from Arrhenius plots (Table I). The data indicated that: (1) the apparent activation energies of the terminal methyl motions were essentially identical in bilayers comprised of PA PhosCho, PO PhosCho, PA PlasCho or PO PlasCho alone; (2) the activation energies of each phospholipid subclass and molecular species decreased with addition of cholesterol; (3) cholesterol had larger effects on the activation energies of the terminal methyl protons in plasmenylcholine bilayers compared to phosphatidylcholine bilayers (e.g., 7.3 kJ/mol for PA PlasCho and 6.7 kJ/mol for PO PlasCho vs. 11.5 kJ/mol for PA PhosCho and 9.1 kJ/mol for PO PhosCho at 40 mol% cholesterol in the bilayer matrix); and (4) the alterations in the activation energies for motion in the terminal methyl groups induced by the addition of cholesterol in PO PhosCho and PO PlasCho were larger than those in PA PhosCho and PA PlasCho. No major differences in the T_1 relaxation times (data not shown) or apparent activation energies (Table I) of aliphatic methylene protons were demonstrated in the absence or in the presence of cholesterol which likely reflects the heterogeneity of molecular environments of different protons that contribute to this resonance.

Dynamics of the polar head group in plasmenylcholine and phosphatidylcholine vesicles

Comparisons of the dynamics of the choline methylene protons ($N\text{-}CH_2$ located in the polar head group) demonstrated that their spin-lattice relaxation times were longer in PA PlasCho bilayers than in PA PhosCho bilayers (Fig. 4A). Similarly, the T_1 values of the choline methylene protons in PO PlasCho bilayers were slightly longer than those present in PO PhosCho (Fig. 4B). Introduction of cholesterol into plasmenylcholine bilayers increased the T_1 relaxation times of the choline methylene protons. In contrast, introduction of cholesterol resulted in only small alterations in the T_1 relaxation times of $N\text{-}CH_2$ protons in both PO PhosCho and PA PhosCho bilayers (Figs. 4A and 4B). Interestingly, the effects of cholesterol on the T_1 relaxation times of

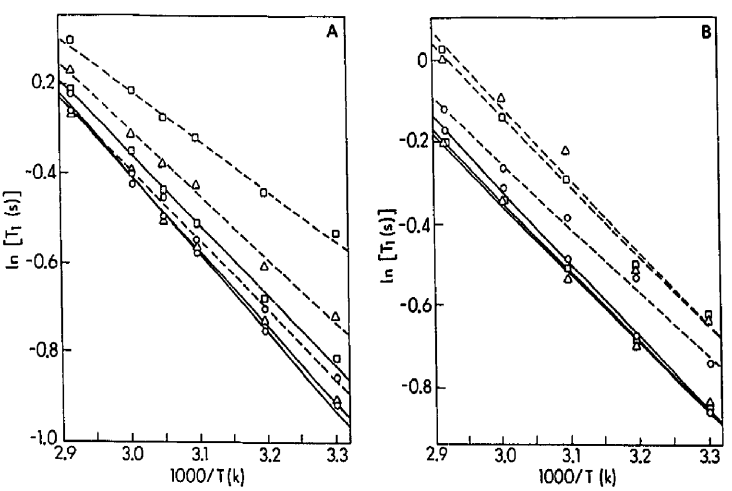

Fig. 4. Arrhenius plots of T_1 relaxation times of choline methylene protons in phosphatidylcholine and plasmenylcholine bilayers containing 0–40 mol% cholesterol. Sonicated unilamellar vesicles of PA PhosCho (———) or PA PlasCho (- - - - -) (A) and PO PhosCho (———) or PO PlasCho (- - - - -) (B) containing 0 (○), 20 (△) or 40 (□) mol% cholesterol were prepared and T_1 relaxation times of choline methylene protons were determined at 300 MHz utilizing the fast inversion recovery method as described in Materials and Methods. Data points are the mean value of at least three independent sample preparations. The standard errors are less than 10% of the mean value.

choline methylene protons, particularly in plasmenylcholine bilayers, were opposite to those on the proton T_1 relaxation times of functional groups located in the membrane interior. The apparent activation energies of choline methylene protons in each subclass at each concentration of cholesterol were similar (Table I). The T_1 relaxation times (data not shown) and the apparent activation energies (Table I) of choline methyl protons ($N\text{-}(CH_3)_3$) in bilayers comprised of each molecular species of plasmenylcholine and phosphatidylcholine examined were nearly identical within experimental error.

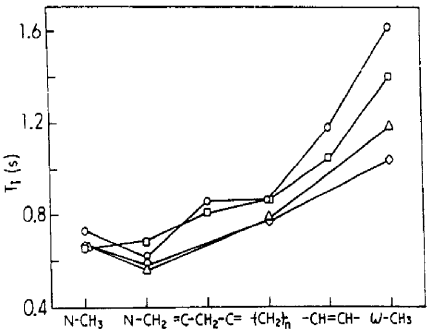

Fig. 5. Comparisons of proton T_1 relaxation times of selected molecular functional groups in phosphatidylcholine and plasmenylcholine bilayers at 50°C. Sonicated unilamellar vesicles of PA PhosCho (○), PA PlasCho (□), PO PhosCho (△) or PO PlasCho (◇) were prepared and proton T_1 relaxation times of selected groups were determined at 300 MHz utilizing the fast inversion recovery method for each functional group as described in Materials and Methods. T_1 data represent the mean value of at least three independent sample preparations. The standard errors are less than 10% of the mean values.

Discussion

Results of the present study demonstrate that significant differences in the spin-lattice relaxation times and activation energies for NMR observable motions are present in plasmenylcholine and phosphatidylcholine subclasses. The spin-lattice relaxation times of vinyl, bisallylic methylene and terminal methyl protons in phosphatidylcholine vesicles are longer than those present in plasmenylcholine vesicles. Furthermore, plasmenylcholine vesicles possess a fluidity gradient (increasing from the outside of the bilayer to the membrane interior) which is smaller than that present in phosphatidylcholine vesicles (Fig. 5). Importantly, the spin-lattice relaxation times of the terminal methyl protons in phospholipids containing arachidonic acid at the *sn*-2 position in both subclasses are substantially longer than that for comparable monoenoic phospholipids. The observed differences in molecular dynamics are also in good agreement with previous ESR studies utilizing spin-labeled phosphatidylcholine and plasmenylcholine probes [14] which demonstrated that molecular motion in the hydrophobic portion of plasmenylcholine is more constrained than in its diacyl phospholipid counterpart. The longer T_1 values of the terminal methyl, vinyl and bisallylic methylene protons present in phosphatidylcholine bilayers compared to plasmenylcholine bilayers likely reflect the tighter packing of plasmenylcholine molecules in membrane bilayers in agreement with a recently proposed model [17].

These studies are also the first to demonstrate that substantial decreases in the proton spin-lattice relaxation times in the interior regions of the plasmenylcholine bilayer result from incorporation of cholesterol into the lipid matrix. Importantly, the magnitude of this decrease is larger in vesicles comprised of plasmenylcholine in comparison to phosphatidylcholine vesicles. Thus, these results demonstrate that each choline glycerophospholipid subclass possesses a differential susceptibility to modulation of their motional characteristics by cholesterol.

The polar head group dynamics (as characterized by the T_1 relaxation times of choline methyl and methylene protons) in bilayers comprised of PO PhosCho or PA PhosCho are not substantially altered by cholesterol which is comparable to results utilizing disaturated phosphatidylcholine in previous studies [18–20]. In contrast, the T_1 relaxation times of choline methylene protons in plasmenylcholine/cholesterol bilayers (both PO PlasCho and PA PlasCho) increase with the introduction of cholesterol. It has previously been demonstrated that cholesterol increases the separation between polar head groups in phosphatidylcholine bilayers thereby attenuating phospholipid–phospholipid interactions [21]. Since the spin-lattice relaxation times of choline methylene protons in plasmenylcholine are substantially increased by cholesterol in comparison to their diacyl phospholipid counterparts, these results are compatible with the 'spacer effect' of cholesterol and support previous conformational studies which demonstrated that plasmenylcholine bilayers are more compact than phosphatidylcholine bilayers at the membrane interface [17].

Typically the ^1H T_1 relaxation time is dependent on both the orientational order and segmental mobility [22]. However, analysis of Arrhenius plots of the temperature dependence of proton spin-lattice relaxation times demonstrated a high coefficient of linearity over the temperature range studied. Such linear behavior is anticipated from the formalisms proposed by Chan et al. [8,24] as well as Brown [22] relating proton T_1 relaxation times to the rotational correlation time in sonicated bilayers. Chan et al. [8,23,24] proposed:

$$1/T_1 = A\tau_{\parallel} + B\omega_o^{-2}\tau_{\perp}^{-1} \qquad (2)$$

where A and B are constants which depend on the nucleus and $\tau_{\parallel}$ and $\tau_{\perp}$ are the effective correlation time parallel and perpendicular to the membrane director, and ω_0 is the observed nuclear magnetic resonance frequency. Similarly, the model proposed by Brown [22] to mathematically describe spin-lattice relaxation time in lipid bilayers is summarized by the following formalism:

$$1/T_1 = A\tau_f + BS_{CH}^2\omega_o^{-1/2} \quad (3)$$

where A, B, and ω_0 have the same meanings as in Eqn. 2, τ_f is the effective correlation time of fast motion and S_{CH} is the observed bond segmental order parameter. At sufficiently high magnetic field and temperature, the second term in Eqns. 2 and 3 can be effectively neglected resulting in the appearance of linear Arrhenius plots. Apparent activation energies obtained from Arrhenius plots of relaxation times identify the temperature-dependent probability of ^{1}H-NMR observable motions and provide additional insight into differences in the membrane dynamics of these subclasses. Smaller activation energies for the motion of bisallylic methylene and vinyl protons in plasmenylcholine bilayers are present in comparison to phosphatidylcholine. Although the activation energies of the terminal methyl protons decrease with incorporation of cholesterol into phosphatidylcholine and plasmenylcholine bilayers, only small effects on other segmental protons are elicited. Importantly, the effect of cholesterol on the activation energies of terminal methyl protons in plasmenylcholine bilayers is much greater than in phosphatidylcholine bilayers. It should be recognized that measurement of spin-lattice relaxation times in unilamellar vesicles are influenced by the constraints present in molecular aggregates which possess a high radius of curvature.

Collectively, these results demonstrate that separate and distinct membrane molecular dynamics and interactions with cholesterol are present in plasmenylcholine and phosphatidylcholine bilayers. Since myocardial sarcolemma is highly enriched in arachidonylated plasmalogens and cholesterol, it is tempting to speculate that specific binary interactions between these lipid constituents facilitate the optimal function of critical transmembrane proteins. The results contained herein underscore the differences between different subclasses of naturally occurring phospholipids. It is hoped that these studies will provide impetus for further ^{1}H spin-spin relaxation experiments and ^{2}H-NMR experiments employing naturally occurring as well as specifically deuterated phospholipids.

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