MOLECULAR DYNAMICS OF VESICLES OF UNSATURATED PHOSPHATIDYLCHOLINES STUDIED BY ^{13}C NMR SPIN-LATTICE RELAXATION

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^{13}C spin-lattice relaxation times, T_{1Z}, were measured at four different magnetic field strengths for a vesicles of a series of unsaturated 1,2-diacyl-*sn*-glycero-3-phosphocholines over the temperature range from 5 to 50 °C. The acyl chains of the phosphatidylcholines varied in length from 14 to 18 carbons and in the degree of unsaturation: myristoleoyl (14 : 1), palmitoleoyl (16 : 1), oleoyl (18 : 1), linoleoyl (18 : 2), linolenoyl (18 : 3). For each of the phosphatidylcholines studied the $R_{1Z} = (NT_{1Z})^{-1}$ values of the acyl chain methylene carbons, where N is the number of directly bonded hydrogens, decreased from the C_2 position which is situated close to the glycerol backbone towards the acyl terminal methyl group. The effect of the double bond was manifested by an increase in R_{1Z} and was less pronounced if it was located closer to the terminal methyl group than in the middle of the chain. A minimum in the T_{1Z} relaxation times was not observed, even for highest frequency of 125.8 MHz and the lowest temperature of 5 °C. In the case of the phosphocholine headgroup and glycerol backbone carbons the T_{1Z} times showed similar temperature and frequency dependencies as the acyl chain carbons. The results of the measurements indicate substantial differences in the dynamics of these systems in the liquid-crystalline state. Analysis of the data using various dynamical models is consistent with a distribution of motions which is not described by a single Lorentzian spectral density. This dispersion appears to be an inherent feature of the dynamics of phosphatidylcholines in the liquid-crystalline state.

Lipids are among the basic structural components of biological membranes and their physical properties may play a role in such phenomena as the transport of non-electrolyte solutes, rotational and translational diffusion of membrane proteins, the activities of membrane proteins, and repulsive bilayer forces involved in membrane fusion. For example, it has been found[1,2], that the photochemical activity of rhodopsin in membranes depends on the lipid headgroup composition and the degree of lipid acyl chain unsaturation. Likewise studies of the Ca^{++}-ATPase of sarcoplasmic reticulum reconsti-

tuted into phosphatidylcholine (PC)* bilayers have indicated[3] that the number of Ca^{++} transported per ATP hydrolyzed (the coupling ratio) depends on the amount of phosphatidylethanolamine (PE) and the degree of unsaturation of the PE acyl chains. The diversity of biomembrane lipids is considerable; yet the role of this lipid diversity in membrane function remains a major unsolved problem in biochemistry. Most studies[4–7] concerned with the dynamical structure of lipid molecules employing NMR spectroscopy have dealt with saturated or monounsaturated lipids in the liquid-crystalline state. Relatively little is known about the dynamics of polyunsaturated lipids[8,9], which are abundant in neural tissues such as cerebral gray matter and the retina, and may be implicated in diseases such as atherosclerosis[10] or cancer[11].

In discussing the molecular properties of lipids in membranes, one must distinguish between their mean-statistical properties, i.e. the time averaged orientation and amplitude of the angular displacements of particular groups within the hydrocarbon chains and headgroup region, and their dynamic properties, i.e. the rates of motion of various segments. Deuterium (^{2}H) NMR and solid state carbon-13 (^{13}C) NMR methods enable one to determine motionaly averaged electric field gradient or dipolar tensors from the spectral lineshapes, and thereby obtain information regarding average conformational properties. Moreover, knowledge of the molecular dynamics of membrane lipids can be obtained through analysis of the corresponding NMR relaxation rates. The latter quantities are related[12] to molecular motions through the irreducible spectral densities of motion, $J_M(\omega)$, which are Fourier transforms partners of the irreducible correlation functions $G_M(\tau)$. The correlation functions $G_M(\tau)$ describe fluctuations in the C–H bond orientation with respect to the static magnetic field B_0 in terms of the second rank tensor components which characterize the quadrupolar or dipolar coupling[13]. Measurements of relaxation times[14–17] using solid-state NMR methods are relatively few in number and are typically restricted to one or at most a few resonance frequencies (magnetic field strengths). Consequently it is difficult to probe the nature of the dynamics in detail. Natural abundance ^{13}C spin-lattice (R_{1Z}) relaxation rate studies of vesicles are advantageous in that they enable data to be obtained over a greater range of field strengths using conventional NMR spectrometers. Such studies are complementary to solid-state NMR methods because little difference is seen in the R_{1Z} rates of vesicles and multilamellar dispersions in the MHz regime in ^{2}H or ^{13}C NMR (ref.[17]). Relaxation

* Abbreviations used: ATP, adenosine triphosphate; di(14 : 0)PC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; di(16 : 0)PC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; di(14 : 1)PC, 1,2-dimyristoleoyl-*sn*-glycero-3-phosphocholine; di(16 : 1)PC, 1,2-dipalmitoleoyl-*sn*-glycero-3-phosphocholine; di(18 : 1)PC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; di(18 : 2)PC, 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine; di(18 : 3)PC, 1,2-dilinolenoyl-*sn*-glycero-3-phosphocholine; EDTA, ethylenediaminetetraacetic acid; MeOH, methanol; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

measurements can provide useful information regarding the molecular dynamics of lipid bilayers in the fluid or in the liquid crystalline state, and can also furnish knowledge of the mobility of membrane lipids that can be compared with results obtained by computer simulations[18–24].

At present, several different motional models[6,25–34] are available for the interpretation of NMR relaxation data, which describe (i) segmental motions, (ii) molecular motions, and (iii) collective excitations of the bilayer assembly. In order to test the applicability of various proposed dynamical models, the spectral densities or relaxation times have to be determined over a broad frequency range. Therefore, a more expanded body of experimental data is needed for construction of realistic motional models for lipid bilayers. In analyzing the NMR relaxation rates of lipids in the liquid-crystalline state, knowledge of their dependence on the length and degree of unsaturation of the acyl chains, as a function of the temperature and the resonance frequency, is clearly desirable. Accordingly, we carried out such extensive studies of the ^{13}C spin-lattice relaxation rates (R_{1Z}) of liquid-crystalline vesicle suspensions of a series of unsaturated and polyunsaturated phosphatidylcholines. We present here the first systematic attention to the effects of the length of the acyl chain, the degree of chain unsaturation, the temperature, and resonance frequency on the ^{13}C R_{1Z} rates. The results of the measurements indicate substantial differences in the dynamics of these systems, and are discussed within the framework of simple models for the molecular dynamics of membrane lipids in the liquid-crystalline state.

EXPERIMENTAL

The 1,2-diacyl-*sn*-glycero-3-phosphocholines were synthesized as described in the literature[35,36]. The symmetric unsaturated phospholipids were composed of acyl chains which varied in length from 14 to 18 carbons and in degree of unsaturation as follows: dimyristoleoyl (14 : 1), dipalmitoleoyl (16 : 1), dioleoyl (18 : 1), dilinoleoyl (18 : 2), dilinolenoyl (18 : 3). The phospholipids were shown to be pure both by thin layer chromatography using a $CHCl_3$–MeOH–H_2O (65 : 35 : 5) solvent system and by gas-liquid chromatography of the deacylated fatty acid methyl esters on 10% SP-2330 (Supelco) using a Varian 3700 gas chromatograph. Thin layer chromatography of the phospholipid samples before and after NMR spectroscopy indicated the absence of any hydrolysis or degradation of the acyl chains.

All manipulations of unsaturated lipid samples were done under a stream of argon. The dry phospholipids were dispersed in 67 mM sodium phosphate buffer (pH 7.0), prepared in 74 vol.% ^2H_2O, containing 0.1 mM EDTA and 0.01 wt.% sodium azide, at a concentration of 300 mg/ml. ^2H_2O served as the frequency/field lock reference, while EDTA removed interfering paramagnetic ions; sodium azide was added to prevent bacterial growth. Each lipid dispersion was equilibrated above the appropriate main order-disorder phase transition temperature, and was sonicated to form small unilamellar vesicles. A Branson W-220F sonicator equipped with a microtip was used at power setting between 2 and 4 units for about two or three 10 min continuous sonication periods, after which a translucent suspension was produced. This preparation was centrifugated (5 000 min^{-1}) for about 5 min to remove titanium particles from the microtip, and then transferred into the NMR tube. All samples were contained under argon in sealed 10 mm NMR tubes and were about 16 mm deep. The

vesicle solutions were never allowed to cool below the corresponding phase transition temperatures until the NMR experiments were completed.

Four different FT NMR spectrometers (JEOL FX-60; Nicolet NT-200, NT-360, and NT-500) were used for this work, operating at magnetic field strengths B_0 (^{13}C resonance frequencies ν_C) of 1.409 (15.04), 4.698 (50.31), 8.479 (90.80), and 11.74 T (125.76 MHz). The sample temperature was measured before and after each experiment using a thermocouple with the ^{1}H-decoupler on. The estimated temperature errors were ± 1 °C. The ^{13}C spin-lattice relaxation times (T_{1Z}) were measured at natural abundance with proton decoupling using the inversion recovery technique[37]. In each case at least fourteen different τ values were used and the waiting period between successive acquisitions was at least 2 – 3 times the longest T_{1Z} time measured. The values of the relaxation times were obtained from three parameter fits to the raw data[38]. The assignment of the lines in the ^{13}C NMR spectrum to individual carbons in a molecule was based on the chemical shift[39–41] δ values and their additivity rules for acyl chains[42,43].

RESULTS

Profiles of ^{13}C R_{1Z} Relaxation Rates as a Function of Acyl Chain Segment Position

The measured spin-lattice relaxation times, T_{1Z}, of the individual carbons represent an average over both lipid acyl chains since distinct lines were not seen (corresponding to a given carbon) for the *sn*-1 and *sn*-2 acyl chains, excepting the carbonyl carbon. Figure 1 shows the dependence of the relaxation rates $R_{1Z} = (NT_{1Z})^{-1}$, where N is the number of protons directly attached to an individual carbon, on the acyl position for vesicles of the

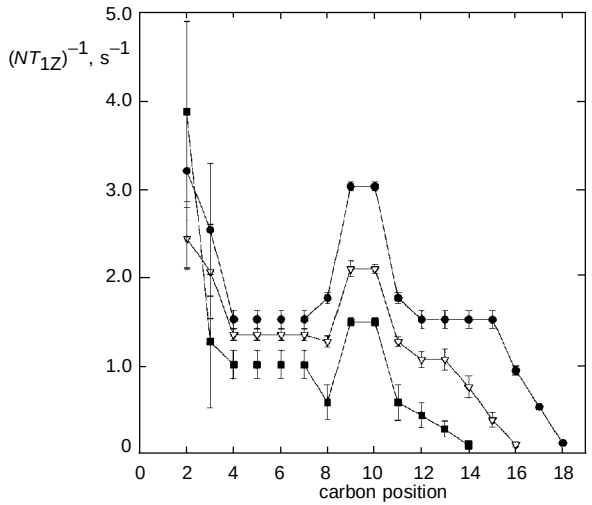

FIG. 1

^{13}C spin-lattice relaxation rates, $R_{1Z} = (NT_{1Z})^{-1}$, as a function of acyl position for vesicles of monounsaturated phosphatidylcholines. The influence of the number of acyl chain carbons is depicted at a constant temperature of 30 °C and resonance frequency of 15.04 MHz (1.409 T); di(14 : 1)PC (■), di(16 : 1)PC (▽), and di(18 : 1)PC (●). Distinct lines were not resolved for acyl chain carbons C_4 to C_7 and C_{12} to C_{15} (di(18 : 1)PC only), for C_8, C_{11}, and for C_9, C_{10}

monounsaturated phosphatidylcholine series, with variable chain length. (Plotting the data as $(NT_{1Z})^{-1}$ accounts for the different numbers of protons of the methine, methylene, and methyl groups. The relaxation rates R_{1Z} enable one to compare directly the dynamics of various segments of the acyl chains.) The data presented in Fig. 1 were obtained at a resonance frequency of $\nu_C = 15.04$ MHz and a temperature $T = 30$ °C. At this temperature, all phosphatidylcholines are in the liquid-crystalline state. For each of the phosphatidylcholines studied, the R_{1Z} values decreased from the C_2 carbon, which is situated close to the glycerol backbone, towards the acyl terminal methyl group located near the middle of the bilayer. The position of the double bond in the acyl chains between carbons C_9 and C_{10} was manifested by a substantial increase of the R_{1Z} values of these olefinic carbons relative to their contiguous neighboring allylic segments (Fig. 1). As is evident, the values of R_{1Z} of each of the methylene and olefinic carbons increased with the number of acyl chain carbon segments. This was typically observed for temperatures measured over a 5 to 50 °C interval, and at all measuring frequencies for the olefinic carbons (C_9, C_{10}) and the bulk of the methylene carbons (C_{11} and beyond). (The increase in the R_{1Z} values with the chain length of monounsaturated phosphatidylcholines was not always observed for the C_2 to C_8 acyl chain methylene carbons.) The relaxation rates of the terminal methyl carbon depended on the chain length, but only weakly.

Influence of Acyl Chain Unsaturation

The effect of acyl chain unsaturation on the relaxation rate R_{1Z} at a constant number of chain carbons is illustrated in Fig. 2. Representative data are shown for vesicles of a series of phosphatidylcholines comprising 18 carbon chains at $T = 15$ °C and $\nu_C = 50.31$ MHz. As for the case of phospholipids having monounsaturated chains (Fig. 1), the R_{1Z} values decreased from the C_2 segment towards the terminal methyl group. The positions of the double bonds in the chains were also clearly manifested by the larger R_{1Z} values of the olefinic carbons compared to their closest neighboring (allylic) segments. However, this difference was found to decrease with an increasing degree of chain unsaturation. The effect of the double bond on the internal mobility of the acyl chains was less pronounced if it was located closer to the terminal methyl group than in the middle of the chain (compare data for oleoyl (18 : 1ω9), linoleoyl (18 : 2ω6), linolenoyl (18 : 3ω3) acyl chains in Fig. 2). In addition, the values of R_{1Z} for the mutually corresponding olefinic and methylene carbons (C_9 to C_{18}) decreased with an increase in the number of double bonds in the chain. This was observed at all the frequencies (15.04 to 125.8 MHz) and temperatures measured (5 to 50 °C). (A decrease in the R_{1Z} values with the increasing degree of chain unsaturation was not always seen for the methylene carbons C_2 to C_8). The relaxation rates of the terminal methyl carbons decreased with increasing unsaturation of the chains.

Effect of Temperature

The effect of temperature on the relaxation rates, R_{1Z}, of the acyl chain carbons is depicted in Fig. 3, which presents data for vesicles of 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine, di(18 : 2)PC, at 90.80 MHz. The R_{1Z} values of all carbons decreased with increasing temperature. For the olefinic carbons, the R_{1Z} rates were again higher than for their closest neighboring (allylic) segments as noted above. Similar results were obtained at all measured frequencies for all the lipids studied. *A maximum in the R_{1Z} values as a function of temperature, i.e. a minimum in the relaxation times, was not observed, even for highest frequency of 125.8 MHz and the lowest temperature of 5 °C.* This is also indicated by Arrhenius plots of the R_{1Z} data, which were linear for each acyl chain carbon (vide infra). It is noteworthy that such T_{1Z} minima have been observed in ^{31}P NMR spin-lattice relaxation studies of the phosphate group of egg yolk phosphatidylcholine multilamellar dispersions[44] and in ^{2}H NMR spin-lattice relaxation studies[45] of [2,2,3,4,4,6-$^{2}H_6$]cholesterol incorporated in model membranes of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine, di(14 : 0)PC.

Representative values of the apparent activation energy, E_a, for the acyl chain carbons of di(18 : 2)PC at ν_C = 90.80 MHz are given in Table I. The lowest value, approximately 10 kJ/mol, was that of the acyl chain carbon C_3. The apparent activation energies of the individual acyl carbons increased towards the terminal methyl group,

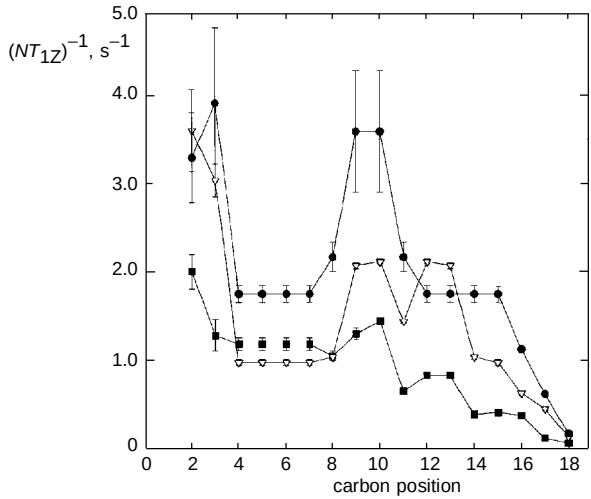

FIG. 2

^{13}C spin-lattice (R_{1Z}) relaxation rates as a function of acyl position for vesicles of monounsaturated phosphatidylcholines. The influence of unsaturation at constant acyl chain length is shown for vesicles of phosphatidylcholines at 15 °C and 50.31 MHz (4.698 T); di(18 : 1)PC (●), di(18 : 2)PC (∇), and di(18 : 3)PC (■). Separate resonances were not observed for acyl chain carbons C_4 to C_7 and C_{12} to C_{15}, for C_8, C_{11}, and for C_9, C_{10} of di(18 : 1)PC; for C_4 to C_7, for C_9, C_{13}, and for C_{10}, C_{12} of di(18 : 2)PC; for C_4 to C_7, and for C_{12}, C_{13} of di(18 : 3)PC

TABLE I

Spin-lattice relaxation times (T_{1Z}) and apparent activation energies (E_a) of carbons of 1,2-dilinolenoyl-*sn*-glycero-3-phosphocholine vesicles at different ^{13}C resonance frequencies (temperature T = 50 °C)

Carbon position[a]	T_{1Z}, s				E_a, kJ mol^{-1}[b]
	15.04 MHz	50.31 MHz	90.80 MHz	125.76 MHz	
C_γ	$0.85_0 \pm 0.06_5$	$0.82_4 \pm 0.03_3$	$0.96_0 \pm 0.02_1$	$0.95_1 \pm 0.01_8$	$22._6 \pm 1._7$
C_β	$0.46_2 \pm 0.05_3$	$0.49_2 \pm 0.05_6$	$0.69_2 \pm 0.03_5$	$0.71_1 \pm 0.02_6$	$22._2 \pm 2._1$
C_α	$0.38_8 \pm 0.03_3$	$0.44_9 \pm 0.09_2$	$0.65_5 \pm 0.02_7$	$0.59_7 \pm 0.02_5$	$20.6 \pm 0.9_1$
C_{sn-3}	$0.06_7 \pm 0.01_5$[c]	$0.07_7 \pm 0.02_4$[c]	$0.18_4 \pm 0.03_9$	$0.19_2 \pm 0.04_8$[c]	$28._6 \pm 1._2$
C_{sn-2}	–	–	$0.29_0 \pm 0.04_6$	–	–
C_{sn-1}	$0.06_7 \pm 0.01_5$[c]	$0.07_7 \pm 0.02_4$[c]	$0.14_6 \pm 0.02_2$	$0.19_2 \pm 0.04_8$[c]	$28._1 \pm 2._3$
C_1[d]	$2.8_0 \pm 0.2_1$	$2.8_9 \pm 0.4_2$	–	$2.6_4 \pm 0.2_4$	–
		$2.9_3 \pm 0.3_4$		$2.9_1 \pm 0.4_9$	
C_2	$0.21_3 \pm 0.02_2$	$0.22_8 \pm 0.09_7$	$0.41_4 \pm 0.03_1$	–	$12._0 \pm 1._2$
C_3	–	$0.4_8 \pm 0.1_2$	$0.58_1 \pm 0.05_3$	–	$10.0 \pm 0.9_4$
C_{4-6}	$0.38_6 \pm 0.04_1$	$0.47_5 \pm 0.04_9$	$0.68_9 \pm 0.05_3$	$0.79_6 \pm 0.02_7$	$12.5 \pm 0.3_5$
C_7	$0.38_6 \pm 0.04_1$	$0.56_3 \pm 0.05_2$	$0.77_1 \pm 0.02_8$	$0.85_0 \pm 0.04_5$	$14._5 \pm 1._1$
C_8	$0.54_5 \pm 0.06_7$	$0.8_5 \pm 0.1_8$	$0.98_4 \pm 0.02_0$	$0.91_3 \pm 0.04_1$	$17.0 \pm 0.1_0$
C_9	$0.97_0 \pm 0.09_5$	$1.1_4 \pm 0.1_0$	$1.53 \pm 0.08_9$	$1.39 \pm 0.07_5$	$17.8 \pm 0.1_4$
C_{10}	–	$1.2_7 \pm 0.05_8$	$1.53 \pm 0.06_7$	$1.54 \pm 0.03_6$	$17.8 \pm 0.1_4$
C_{11}	$1.47 \pm 0.04_2$	$1.7_1 \pm 0.1_7$	$1.77 \pm 0.04_7$	$2.60 \pm 0.04_1$	$19.5 \pm 0.1_7$
C_{12-13}	–	$2.21 \pm 0.06_5$	$2.64 \pm 0.09_2$	$2.59 \pm 0.08_6$	$19._6 \pm 2._6$
C_{14}	$1.7_0 \pm 0.1_7$	$2.57 \pm 0.08_8$	$2.78 \pm 0.09_3$	$2.6_4 \pm 0.1_0$	$19._7 \pm 3._8$
C_{15}	–	$4.1_9 \pm 0.1_0$	$5.1_3 \pm 0.2_2$	$4.8_1 \pm 0.1_1$	$19._4 \pm 1._2$
C_{16}	$4.6_2 \pm 0.4_8$	$4.9_6 \pm 0.2_1$	$5.9_3 \pm 0.1_3$	$5.5_5 \pm 0.2_3$	$19._4 \pm 1._2$
C_{17}	$5.6_4 \pm 0.9_6$	$6.7_8 \pm 0.3_3$	$8.5_7 \pm 0.3_1$	$8.6_8 \pm 0.6_8$	$20._5 \pm 1._9$
C_{18}	$6.6_9 \pm 0.9_8$	$7.5_6 \pm 0.3_0$	$8.2_2 \pm 0.4_4$	$8.4_8 \pm 0.9_4$	$15._9 \pm 2._3$

[a] See footnote for definition of carbon segment. [b] Resonance frequency of 90.80 MHz. [c] Distinct lines were not observed for *sn*–1 and *sn*–3 segments. [d] Separate lines were observed for *sn*–1 and *sn*–2 chains except at the lowest frequency of 15.04 MHz.

reaching a maximum of 20.5 kJ/mol for C_{17}. In the liquid-crystalline state, somewhat larger values of E_a are found for the glycerol backbone and polar head group carbons. The values of the apparent activation energy E_a given in Table I are in the range determined from ^{2}H NMR spin-lattice relaxation[46–48] for fast *trans–gauche* isomerization motions. However, such a conventional interpretation of the temperature dependence of the R_{1Z} rates in terms of an activation energy E_a may not be applicable if the relaxation is govern by slower order fluctuations (vide infra). The results could then be explained in terms of temperature effects on the Boltzman distribution which governs the effective or residual dipolar coupling remaining from the faster segmental rotations[30].

Frequency Dependence of Spin-Lattice (R_{1Z}) Relaxation Rates

The variation of the spin-lattice relaxation times T_{1Z} with the ^{13}C resonance frequency (magnetic field strength) is summarized in Tables I and II for di(18 : 2)PC and di(14 : 1)PC. It is evident that the T_{1Z} values of the individual acyl chain carbons increased with increasing frequency, ν_C, both for the polyunsaturated (Table I) and monounsaturated (Table II) phosphatidylcholines in the liquid-crystalline state. There was a greater increase with frequency for the T_{1Z} times of carbons closer to the glycerol backbone. Towards the terminal methyl group, the dependence of T_{1Z} on ν_C became weaker, except for the olefinic carbons. The effect of the double bond was more pronounced if it was situated

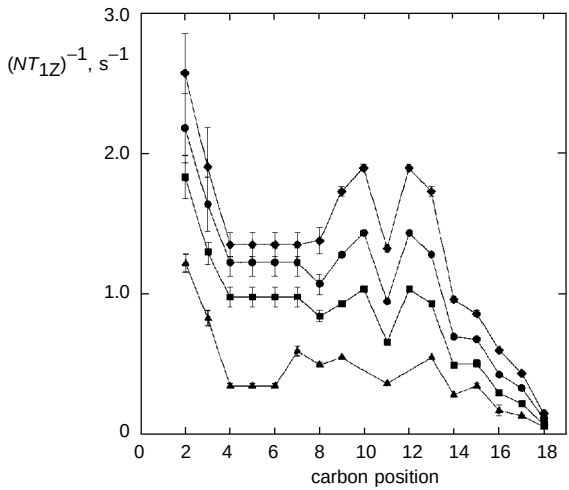

FIG. 3
^{13}C spin-lattice (R_{1Z}) relaxation rates as a function of acyl position for vesicles of 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine. The influence of temperature at 90.80 MHz (8.479 T) is illustrated; 5 °C (◆), 15 °C (●), 30 °C (■), and 50 °C (▲). Distinct resonances were not observed for acyl chain carbons C_4 to C_7, for C_9, C_{13}, and for C_{10}, C_{12} at 5, 15, and 30 °C; and for C_4 to C_6 at 50 °C

in the middle of the chain than if it was closer to the terminal methyl group. In the case of the methyl group itself there was relatively small dependence of T_{1Z} on ν_C. The dependence was also less pronounced at higher temperatures and for the polyunsaturated versus the monounsaturated series of phosphatidylcholines.

Phosphocholine Headgroup Segments

The spin-lattice relaxation times T_{1Z} of the phosphocholine headgroup carbons* showed similar temperature and frequency dependencies as the acyl chain carbons (Tables I, II). The values of their activation energy, E_a, were also similar to the values for the acyl carbons close to the terminal methyl group (Tables I, II). We have found that for a particular temperature and frequency, the T_{1Z} times of the C_γ and C_β carbons of the phosphocholine headgroups do not depend greatly on the length or degree of acyl chain unsaturation. An effect of the length and degree of unsaturation was only seen on the relaxation times of the C_α headgroup carbon and glycerol backbone carbons. However, the measured values of T_{1Z} did not show any monotonous dependency.

Glycerol Backbone Segments

The spin-lattice relaxation times of the glycerol backbone carbons were significantly smaller than those of the headgroup and acyl chains at a particular temperature and frequency (Tables I, II). This fact demonstrates that the glycerol backbone is the most rigid part of the phosphatidylcholine molecule. The glycerol carbons also evince a similar frequency and temperature dependence as the acyl chain carbons.

DISCUSSION

Current knowledge indicates that measurement of the nuclear spin-lattice relaxation rates of membrane lipids can provide novel and important information regarding their molecular dynamics in liquid-crystalline assemblies, viz. vesicles or multilamellar dispersions. In this regard the ^{13}C nucleus is particulary well suited because of its wide range of chemical shifts; upon ^1H decoupling only one line in the NMR spectrum belongs to each chemically non-equivalent carbon. Well resolved ^{13}C NMR spectra of vesicles of disaturated phosphatidylcholines are obtained[14]; moreover application of

* The following designation of the carbon atoms of phosphatidylcholines is used in this article. Headgroup carbons: C_α, OCH_2; C_β, CH_2N; C_γ, $N(CH_3)_3$. Glycerol backbone carbons: C_{sn-1}, OCH_2; C_{sn-2}, OCH; C_{sn-3}, CH_2OP. Acyl chain carbons: C_1, C=O; C_i, CH_2 or CH where $i = 2,...,n-1$; C_n, terminal CH_3. The lipid notation is di(X : Y)PC, where X is the number of carbon atoms and Y the number of double bonds of the acyl chains, and PC denotes the phosphocholine headgroup.

solid-state magic angle spinning ^{13}C NMR techniques to phosphatidylcholine dispersions yields similarly well resolved spectra[49]. Consequently, values of the spin-lattice relaxation time T_{1Z} can be measured for many of the distinct carbons within a membrane lipid bilayer without the necessity of isotopic labeling. The relaxation of the ^{13}C nucleus is predominantly determined by the ^{13}C–^{1}H direct dipolar coupling; this interaction is a random function of time because the orientation of the ^{13}C–^{1}H vector fluctuates stochastically with respect to the external magnetic field. Interpretation of the observed quantities necessitates the introduction of models for the dynamical processes underlying the relaxation.

TABLE II
Spice-lattice relaxation times (T_{1Z}) for carbons of 1,2-dimyristoleoyl-*sn*-glycero-3-phosphocholine vesicles at different ^{13}C resonance frequencies (temperature T = 15 °C)

Carbon position[a]	T_{1Z}, s			
	15.04 MHz	50.31 MHz	90.80 MHz	125.76 MHz
C_γ	0.261 ± 0.007	0.238 ± 0.004	0.305 ± 0.006	$0.37_3 \pm 0.01_1$
C_β	$0.11_3 \pm 0.01_1$	$0.15_7 \pm 0.01_7$	0.231 ± 0.008	$0.30_4 \pm 0.02_4$
C_α	$0.09_4 \pm 0.01_3$	$0.15_6 \pm 0.03_5$	0.224 ± 0.008	$0.28_5 \pm 0.01_3$
C_{sn-3}	0.035 ± 0.009[b]	$0.03_6 \pm 0.01_5$[b]	$0.07_7 \pm 0.01_5$	0.12 ± 0.01[b]
C_{sn-2}	–	–	$0.13_7 \pm 0.06_4$	–
C_{sn-1}	0.035 ± 0.009[b]	$0.03_6 \pm 0.01_7$[b]	$0.11_8 \pm 0.04_1$	0.12 ± 0.01[b]
C_1[c]	$1.3_1 \pm 0.1_1$	$1.8_0 \pm 0.2_3$	–	$2.0_1 \pm 0.3_9$
		$1.5_7 \pm 0.1_5$		$2.6_5 \pm 0.7_2$
C_2	0.102 ± 0.006	$0.13_3 \pm 0.01_5$	$0.22_9 \pm 0.01_1$	$0.31_6 \pm 0.06_1$
C_3	$0.15_2 \pm 0.01_8$	$0.13_7 \pm 0.02_1$	$0.22_7 \pm 0.01_3$	–
C_{4-7}	0.182 ± 0.009	$0.23_1 \pm 0.01_2$	0.351 ± 0.009	$0.45_1 \pm 0.03_2$
C_8	$0.39_4 \pm 0.02_6$	$0.28_6 \pm 0.02_2$	$0.40_6 \pm 0.01_0$	$0.50_3 \pm 0.03_7$
C_{9-10}	$0.40_0 \pm 0.01_1$	$0.41_7 \pm 0.01_0$	$0.55_2 \pm 0.01_5$	$0\ 67_9 \pm 0.01_2$
C_{11}	$0.39_4 \pm 0.02_6$	$0.28_6 \pm 0.02_2$	0.626 ± 0.009	$0.81_9 \pm 0.03_9$
C_{12}	$0.59_7 \pm 0.03_7$	$0.67_4 \pm 0.02_3$	$0.89_1 \pm 0.01_3$	$1.16_6 \pm 0.04_7$
C_{13}	$0.95_9 \pm 0.05_4$	$0.99_5 \pm 0.02_3$	$1.25 \pm 0.01_5$	$1.65_6 \pm 0.04_1$
C_{14}	$1.80_0 \pm 0.06_8$	$1.87_8 \pm 0.05_7$	$2.21 \pm 0.02_8$	$2.8_7 \pm 0.1_9$

[a] See footnote for definition of carbon segment. [b] Distinct lines were not observed for *sn*–1 and *sn*–3 segments. [c] Separate lines were observed for *sn*–1 and *sn*–2 chains except at the lowest frequency of 15.04 MHz.

In the MHz regime the spin-lattice relaxation rates (R_{1Z}) of small vesicles and multilamellar dispersions are nearly identical, so that the results can be compared directly[46,49]. Here we present measurements of the temperature and resonance frequency dependencies of the ^{13}C spin-lattice relaxation times of vesicles of phosphatidylcholines having monounsaturated and polyunsaturated chains of different lengths. These results suggest that the mobility of the acyl groups is not determined by a single type of molecular motion, but rather is complex. There exists a relaxation rate gradient along the chains for both the methylene and olefinic segments, as is evident from the dependence of R_{1Z} on the carbon segment position. We have shown that the gradient monotony is interrupted at the double bond position; for monounsaturated chains the effect of the double bond increases with the chain length. Furthermore, in polyunsaturated chains (for constant acyl length), the HC=CH segments have a relaxation profile along the chain that is analogous to that of the CH_2 groups of saturated phospholipids. Thus the effect of double bond closest to the middle of the chain is most pronounced and gradually decreases for the other double bonds closer to the terminal methyl group.

As a means of accounting for the observed temperature and frequency dependencies of the spin-lattice relaxation rates, one can first consider fast segmental motions described in terms of discrete jumps[48] or alternatively continuous rotational diffusion within an ordering potential[30]. As another possibility, a model has been proposed[6] which attributes the slow relaxation dispersion to the one-dimensional diffusion of rotameric defects such as kinks ($g\pm tg\mp$) along the hydrocarbon chain. Such a model predicts an $\omega_0^{-1/2}$ frequency dependence in the MHz regime given that $\omega_0\tau_b << 1 << \omega_0\tau_d$, where $\omega_0 = 2\pi\nu_0$ is the Larmor frequency. The correlation time τ_b is the mean diffusion time which a defect needs to pass a segment, and the correlation time τ_d is the mean diffusion time which a defect takes to diffuse from one polar headgroup to the opposite within the bilayer. The additional influences of slow fluctuations in the local ordering (order fluctuations) can also be considered[30–32]. Three broad classes of motions of lipid molecules which describe the effect of slow fluctuations in the local ordering set up by fast internal segmental motions have been suggested. The first (i) is a simple rotational diffusion model[30,50] in which the slow motions can be approximated in terms of a Lorentzian-type dispersion law. In the second type of model, (ii) the relatively slow order fluctuations are formulated in terms of motion of a local director axis and are viewed to be intrinsically long range in nature, extending over several segmental diameters. The director fluctuations are described by a continuous distribution of elastic or first-order relaxation modes, which can be approximated within the free membrane limit by an $\omega_0^{-1/2}$ dependence[28,30]. By analogy with nematic and smectic liquid crystals, such motions are termed order-director fluctuations (ODF). Alternatively (iii) the reduced dimensionality of smectic or lamellar systems can be considered[33,51], which is analogous to a smectic undulation model[51] and predicts an ω_0^{-1} dependence. Let us now consider the formulation of the various models in greater depth.

In general, application of the Bloch–Wangsness–Redfield theory[52] to relaxation arising from orientational fluctuations in the $^{13}C–^{1}H$ direct dipolar interactions yields Eq. (*1*),[31,32]

$$R_{1Z} \equiv 1/NT_{1Z} = 3/2\ \pi^2\chi^2[1/6\ J_0(\omega_H - \omega_C) + 1/2\ J_1(\omega_C) + J_2(\omega_H + \omega_C)]\ , \qquad (1)$$

in which R_{1Z} is the spin-lattice relaxation rate, T_{1Z} is the corresponding spin-lattice relaxation time, and N the number of protons directly bonded to the ^{13}C nucleus. Here $\chi \equiv (\gamma_H\gamma_C h/2\pi^2)\langle r_{CH}^{-3}\rangle$ is the heteronuclear dipolar coupling constant; the averaging is over fast vibrational coordinates such that $\langle r_{CH}^{-3}\rangle^{-1/3} \approx 1.14$ Å for ^{13}C NMR (ref.[32]). The $J_M(\omega)$ are irreducible spectral densities[52], where $M = 0, \pm1$, and ±2, and ω is the angular frequency; $\omega_H = 2\pi\nu_H$ and $\omega_C = 2\pi\nu_C$ are the Larmor frequencies for ^{1}H and ^{13}C, respectively. The spectral densities are Fourier transform partners of the correlation functions $G_M(t)$ for the orientational fluctuations of the $^{13}C–^{1}H$ bonds, and are given by Eq. (*2*)

$$J_M(\omega) = \mathrm{Re}\int_{-\infty}^{+\infty} G_M(t)e^{-i\omega t}dt\ , \qquad (2)$$

where

$$G_M(t) = \langle D_{0M}^{(2)*}(\Omega;0)\ D_{0M}^{(2)}(\Omega;t)\rangle - |\langle D_{0M}^{(2)}(\Omega)\rangle|^2\ \delta_{0M}\ . \qquad (3)$$

In Eq. (*3*) $D_{0M}^{(2)}(\Omega)$ are elements of the Wigner rotation matrix and $\Omega(t)$ are the Euler angles which rotate the molecule-fixed principal axes of the dipolar coupling tensor to the laboratory frame of the main magnetic field B_0, i.e. $\Omega(t)$ describes fluctuations of the $^{13}C–^{1}H$ bond orientation with respect to B_0 which produce the relaxation.

Now in phospholipid bilayers and liquid crystals the motions are restricted in their angular amplitude with respect to a preferred director axis, about which there is cylindrical symmetry on average. This leads to a characteristic orientation dependence of the relaxation[30,53]. However, for vesicles of phospholipids or other isotropic systems, the dependence of the spectral densities on the projection index M vanishes due to the spherical averaging, such that $J_M(\omega) \rightarrow \langle J_M(\omega)\rangle \equiv J(\omega)$. Accordingly one obtains Eq. (*4*)

$$R_{1Z} = 3/2\ \pi^2\chi^2\ [1/6\ J(\omega_H - \omega_C) + 1/2\ J(\omega_C) + J(\omega_H + \omega_C)]\ . \qquad (4)$$

For liquid-crystalline assemblies of phospholipids, one can expect a hierarchy of motions, in which local segmental isomerizations of the chains modulate the static $^{13}C–^{1}H$ dipolar coupling. Slower motions can then further average the residual dipolar coupling due to the local ordering, thus yielding an additional relaxation contribution. The presence and magnitudes of the various putative relaxation contributions have been debated. Given that both fast and slow motions are present, for the case of phospholipid vesicles one can write Eq. (*5*)

$$J(\omega) = J_f(\omega) + J_s(\omega) \quad , \tag{5}$$

where $J(\omega)$ is the observed spectral density and $J_f(\omega)$ and $J_s(\omega)$ are the spectral densities due to fast and slow motions, respectively. (Statistically independent fluctuations yielding time-scale separation are assumed, so that cross-correlations are neglected.)

Assuming a single correlation time for the fast motions yields[31] Eq. (*6*)

$$J_f(\omega) = 1/5\ [1 - S_f^{(2)2}]\ j_f^{(2)}(\omega) \quad , \tag{6}$$

where

$$j_f^{(2)}(\omega) = 2\ \tau_f^{(2)} / (1 + [\omega\ \tau_f^{(2)}]^2) \quad . \tag{7}$$

For ^{13}C relaxation due to fluctuating dipolar interactions $\omega = (\omega_H - \omega_C)$, ω_C, or $(\omega_H + \omega_C)$; $j_f^{(2)}(\omega)$ are the reduced spectral densities for the fast motions; and $\tau_f^{(2)}$ is the correlation time for the random motions with respect to the local director axis (internal frame). Note if only a single type motion is considered then $\tau_f^{(2)} \rightarrow \tau^{(2)}$ in Eqs (*6*) and (*7*).

In the case of the relaxation due to slow motions, one can consider a non-collective molecular model, or alternatively a continuum model for collective fluctuations of the bilayer as mentioned above. The former assumes that the slow motions can be treated in terms of discrete modes which correspond to rotations of the flexible molecules, e.g. involving reduced or averaged moments of inertia. In the latter case such a discrete picture is relinquished, so that the slow motions represent a broad distribution of fluctuations due to collective excitations formulated in terms of wave-like modes.

It is simple to show that the results for the non-collective model are isomorphous to those given above for the case of fast motions[30]. Here the spectral densities are scaled by the fast order parameter squared, viz. $S_f^{(2)2}$. Assuming a single correlation time one obtains[31] Eq. (*8*)

$$J_s(\omega) = 1/5\ S_f^{(2)2}\ [1 - S_s^{(2)2}]\ j_s^{(2)}(\omega)\ , \qquad (8)$$

where

$$j_s^{(2)}(\omega) = 2\ \tau_s^{(2)}/(1 + [\omega\tau_s^{(2)}]^2)\ . \qquad (9)$$

Note that if the correlation time for the slow motions is sufficiently long that $[\omega\tau_s^{(2)}]^2 >> 1$, then an ω^{-2} frequency dependence results[4]. By contrast, for the collective model a continuous distribution of modes is present, in which the high frequency cutoff at the segmental dimensions is neglected. Within the free membrane limit a three-dimensional formulation of the local director fluctuations in terms twist, splay, and bend modes[30,31] yields Eq. (*10*):

$$J_s(\omega) = 1/5\ S_f^{(2)2}\ j_s^{(2)}(\omega)\ . \qquad (10)$$

Here

$$j_s^{(2)} = C\ \omega^{-1/2}\ , \qquad (11)$$

in which C is a collection of elastic and other constants. Alternatively, the strongly coupled regime can be considered in which the finite thickness of the bilayer is neglected[33]. Such a formulation in terms of splay only gives[33,51] Eq. (*12*):

$$j_s^{(2)} = D\ \omega^{-1}\ , \qquad (12)$$

where D is similarly a collection of elastic and other constants.

The above limiting results for the frequency dependence of the spin-lattice relaxation rates of lipid vesicles in the MHz regime can be summarized by a power law of the form[46] of Eq. (*13*)

$$R_{1Z} = a\ \omega_0^{-b} + c\ , \qquad (13)$$

where ω_0 (= ω_C) is the Larmor frequency and *a*, *b*, *c* are adjustable parameters. The theoretically predicted values for *b* are 2 (Lorentzian or non-collective model in the long correlation time regime), 1/2 (three-dimensional collective and defect diffusion models), and 1 (interfacial collective model). The results of nonlinear regression fitting of the ^{13}C R_{1Z} values of each of the resolved acyl chain resonances of the phosphatidylcholine vesicles to Eq. (*13*) holding the exponent *b* fixed at 2 suffices to exclude the simple Lorentzian non-collective model (i) in the long correlation time limit as an appropriate description of the frequency dependence of the spin-lattice relaxation times (results not shown). Due to the limited range of frequencies investigated, the experimental R_{1Z} values can be fit with either an $\omega_C^{-1/2}$ or ω_C^{-1} dependence. Although the $\omega_C^{-1/2}$ dependence fits the R_{1Z} data better at higher temperatures (≈50 °C), the ω_C^{-1} dependence does better at lower temperatures (≈15 °C). However[16,46], a model for collective slow motions formulated in terms of a continuous distribution of correlation times with an $\omega_0^{-1/2}$ dispersion law best describes more extensive frequency dependent ^{13}C and ^2H relaxation data for the acyl CH_2 groups of vesicles of di(14 : 0)PC and di(16 : 0)PC in the liquid crystalline state. The currently available R_{1Z} data for vesicles of unsaturated phosphatidylcholines do not show an unambiguous frequency dependence that is ascribable to order fluctuations, on account of the smaller range of magnetic field strengths investigated. In addition the contribution from order fluctuations, i.e. due to non-collective or collective motions, may be significantly smaller with regard to the spin-lattice relaxation of the HC=CH segments[30]. Because of the larger volume of these segments versus the CH_2 groups, the local motions of the olefinic groups are slower than in the case of the methylene groups, leading to a larger local spectral density contribution near the Larmor frequency. On the other hand, due to geometrical effects the order parameters are decreased indicating a smaller contribution from slow motions due to order fluctuations; further work is necessary. It follows that the detailed R_{1Z} relaxation profiles in the case of vesicles of unsaturated phosphatidylcholines may reflect substantial contributions from both local segmental motions of the HC=CH groups, as well as order fluctuations in the case of the acyl CH_2 groups. To conclude, it is plausible to suggest that in addition to local segmental motions collective excitations of membrane assemblies are a general feature of their dynamical behavior[14].

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