Carbon-13 Nuclear Magnetic Resonance Studies of Short-Chain Lecithins. Motional and Conformational Characteristics of Micellar and Monomeric Phospholipid†

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ABSTRACT: ^{13}C NMR spectra have been obtained at 67.9 MHz for several synthetic short-chain phosphatidylcholines: monomeric dibutyrylphosphatidylcholine and dihexanoylphosphatidylcholine (in CD_3OD) and micellar dihexanoyl-, diheptanoyl-, and dioctanoylphosphatidylcholine. Using this series of lecithins, we compare the structure and motion of phosphatidylcholine as a monomer and in micellar environments of widely varying sizes. Resonances for every carbon in each compound are resolved. In particular, the *sn*-1 and *sn*-2 fatty acyl chains are observed to be magnetically nonequivalent. The extent of this chain nonequivalence is a function of the phospholipid aggregation state: for monomeric lipids only the α carbons show a chemical-shift difference (12 Hz), while in micelles the α, β, γ, and, frequently, δ carbons are split (7, 9, 9, and 4 Hz, respectively). Spin–lattice relaxation times also show different behavior for monomer and micellar lipid. For monomers, relaxation times of the alkyl carbons increase monotonically from glycerol backbone to terminal methyl group, and the nonequivalent *sn*-1 and *sn*-2 α-methylene carbons have identical T_1 relaxation times. For phosphatidylcholine micelles, T_1 values of the *sn*-1 and *sn*-2 α- and β-methylene carbons are significantly different, the *sn*-2 chain possessing shorter relaxation times. Rather than a monotonic increase in T_1 as predicted by several theoretical treatments, micellar lipids tend to show similar values for all but the last two carbons. The ^{13}C spin–lattice relaxation studies of phosphatidylcholine micelles are compared with ^2H NMR relaxation studies of dipalmitoylphosphatidylcholine bilayers. We suggest that similar phospholipid motions contribute to the relaxation times of ^{13}C nuclei in micelles and ^2H nuclei in bilayers. These ^{13}C NMR studies suggest that a change in monomer phospholipid conformation/orientation occurs as the molecule becomes packed in an interface. Changes in the phospholipid conformation may be critical to the interfacial activation exhibited by several water-soluble phospholipases toward these short-chain lecithins.

The conformation and dynamic behavior of biological and model membranes are dictated by the structure of their constituent phospholipids. We have begun a study of synthetic short-chain phosphatidylcholines in which the fatty acyl chains have four to eight carbon atoms. Unlike long-chain phospholipids which form bilayers when dispersed in aqueous solutions, these short-chain lecithins exist as monomers [below a critical micelle concentration (cmc)[1]] or large micellar aggregates whose size depends on fatty acyl chain length (Tausk et al., 1974a–c). Often they can substitute a requirement for long-chain phospholipids: they have been used to activate and extract membrane-bound enzymes (Baron & Thompson, 1975; Gazzotti et al., (1975) and to reconstitute lipoprotein complexes (Reynolds et al., 1977). Although as monomers these lecithins can serve as substrates for several water-soluble phospholipases, there is a large rate enhancement for micellar phospholipid (Verger & de Haas, 1976; de Haas et al., 1977). One possible explanation for this "interfacial activation" is that upon aggregation there is an alteration of phospholipid conformation/orientation to a form more easily hydrolyzed (Wells, 1974) . Thus, a detailed study of the conformation and motion of these short-chain phospholipids may shed light on the influence of phospholipid structure on membrane function and protein–lipid interactions.

We have chosen natural abundance ^{13}C NMR to characterize the conformation and dynamics of these short-chain phosphatidylcholines. Magnetic resonance has proven to be a powerful tool for studying phospholipid conformation and motion in lipid bilayers (Horwitz et al., 1972; Seelig & Niederberger, 1974; Yeagle, 1978; Birdsall et al., 1972; Smith, 1979) and in phospholipid/detergent mixed micelles (Ribeiro & Dennis, 1975; Roberts & Dennis, 1978). ^{13}C NMR is particularly promising for studying the motion of alkyl chains because it is sensitive to segmental motions and coupled isomerizations about carbon–carbon bonds (London & Avitabile, 1977; Levy et al., 1977). Resonances for methylene carbon atoms in the center of the alkyl chain in long-chain lecithins form an unresolved envelope (Sears, 1975), although specific labeling with ^{13}C at a few positions has yielded some relaxation data (Lee et al., 1976). In contrast, we find that for the short-chain phosphatidylcholines all carbons are resolvable at 67.9 MHz. This enables us to study chemical shifts and spin–lattice relaxation times (T_1) of each carbon as a function of phospholipid length and aggregation state.

For micelles, carbon atoms in equivalent positions in the *sn*-1 and *sn*-2 chains are magnetically nonequivalent as far as four chemical bonds from the ester carbonyl and can show significantly different relaxation times. For monomer phosphatidylcholine, only the α-methylene carbons are nonequivalent, although the two T_1 s are the same. These results are compared with ^2H NMR relaxation studies of dipalmitoylphosphatidylcholine bilayers.

Experimental Procedure

Materials. Dibutyrylphosphatidylcholine (dibutyryl-PC) and dihexanoylphosphatidylcholine (dihexanoyl-PC) were obtained from Calbiochem and Supelco, Inc.; diheptanoylphosphatidylcholine (diheptanoyl-PC) and dioctanoyl-

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[1] Abbreviations used: cmc, critical micelle concentration; diacyl-PC, 1,2-diacyl-*sn*-glycero-3-phosphorylcholine; T_1, spin–lattice relaxation time; NOE, nuclear Overhauser effect; Me_4Si, tetramethylsilane.

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phosphatidylcholine (dioctanoyl-PC) were obtained from Supelco, Inc. Phospholipid stability was monitored throughout the study by thin-layer chromatography in $CHCl_3$/CH_3OH/H_2O (65:24:4) or $CHCl_3$/CH_3OH/10.4 M NH_4OH (60:35:8) and by ^{1}H NMR analysis at 270 MHz of the α-methylene region (Roberts et al., 1978). Lipid concentrations (45–70 mM) were determined by phosphate analysis (Eaton & Dennis, 1976) and ^{31}P NMR spectroscopy using phosphate buffer as reference (Roberts et al., 1979).

Lipid samples were prepared for NMR by removal of the organic storage solvent under a stream of nitrogen, lyophilization, and solubilization in an appropriate solvent (0.05 M sodium phosphate and 0.005 M EDTA, pD 7.9, or CD_3OD). NaCl (1.0 M) was added to aqueous dihexanoyl-PC samples to lower the cmc from 14 to 8 mM, and so minimize the contribution of monomer to the chemical shifts and relaxation parameters (Tausk et al., 1974a). The dioctanoyl-PC sample contained 0.2 M KSCN to stabilize the micellar phase (Tausk et al., 1974b).

Synthesis of 1-Hexanoyl-2-perdeuteriohexanoyl-sn-glycero-3-phosphorylcholine. Dihexanoyl-PC was solubilized in 0.05 M sodium borate and 0.005 M $CaCl_2$, pH 7.9. Cobra venom phospholipase A_2 (20.4 units) covalently linked to polyacrylamide gel particles (M. F. Roberts, A. Pollak, and G. M. Whitesides, unpublished results) was added to form the lyso compound. The pH was readjusted with 0.5 M NaOH as needed throughout the reaction. The phospholipase was removed by centrifugation at 6800*g* for 10 min. The sample was lyophilized and subsequently extracted with $CHCl_3$/CH_3OH (2:1) to separate inorganic salts from fatty acid and lyso compound. Reacylation of 1-hexanoyllyso-PC was achieved using a fivefold excess of the fatty acid imidazolide of perdeuteriohexanoic acid (Merck Isotopes, Inc.) (Boss et al., 1975). The compound 1-hexanoyl-2-perdeuteriohexanoyl-PC was purified by silicic acid column chromatography. Deuterium incorporation was checked by analyzing the ^{1}H NMR spectrum in CD_3OD.

NMR Spectroscopy. ^{13}C NMR measurements were made at 67.9 MHz with a Bruker 270 spectrometer equipped with a Nicolet 1080 data system. Chemical shifts are reported relative to the external Me_4Si in $CDCl_3$. Relevant spectrometer characteristics are: quadrature phase detection, 90° pulse width of 25–27 μs, sample tube diameter of 10 mm, and ambient operating temperature of 303 K (unless otherwise noted).

Spin–lattice relaxation times were measured by the inversion recovery method, using a recycle time 4–5 times the longest T_1 (Vold et al., 1968). T_1 values were determined by a least-squares fit to $S(t) = S_\infty(1 - 2e^{-t/T_1})$, where $S(t)$ is the amplitude of the carbon peak for time t between the 180 and 90° pulses. Points where $S(t) \geq 0.75S_\infty$ were excluded. The uncertainty in T_1 was found from $\sigma(T_1) = \sigma(1/T_1)T_1^2$, where $\sigma(1/T_1)$ is the uncertainty in $(1/T_1)$ found from the least-squares fit. Each T_1 and its standard deviation were determined from a weighted average of at least three relaxation measurements (Cruickshank, 1965). Nuclear Overhauser enhancements (NOE) were measured by gated proton decoupling (Freeman et al., 1972).

Results

Chemical-Shift Assignments. Short-chain lecithins have four to eight carbon length fatty acyl chains. The alkyl chain carbons are labeled α (methylene carbon adjacent to the carbonyl), β (methylene carbon adjacent to α), γ, δ . . . $\omega - 1$ (penultimate carbon), and ω (terminal methyl). Cmc's of 8, 1.4, and 0.25 mM and approximate mean aggregation numbers of 32, 150, and 8100 for dihexanoyl-PC, diheptanoyl-PC and dioctanoyl-PC micelles, respectively, have been determined previously (Tausk et al., 1974a) for the concentrations of phospholipids used in this ^{13}C NMR study.

Table I: ^{13}C NMR Chemical Shifts (Parts per Million from External Me_4Si) for Short-Chain Phosphatidylcholines

	monomer		micelle		
carbon atoms	dibutyryl-PC	dihexanoyl-PC (CD_3OD)	dihexanoyl-PC	diheptanoyl-PC	dioctanoyl-PC
backbone					
CHO	70.83	71.03	70.66	70.71	70.67
	70.74	70.94	70.55	70.62	70.58
CH_2OP	63.96	64.68	63.70	63.76	63.73
CH_2O	62.60	62.79	62.91	63.00	63.04
head group					
CH_2N	66.08	66.65	66.01	66.04	66.10
CH_2OP	59.55	60.21	59.45	59.49	59.58
+					
$N(CH_3)_3$	54.04	54.04	54.04	54.07	54.13
alkyl					
α (*sn*-2)	35.85	34.34	33.91	34.10	34.14
(*sn*-1)	35.68	34.14	33.80	34.00	
β (*sn*-2)		24.95	24.34	24.83	24.98
(*sn*-1)			24.23	24.68	24.83
γ (*sn*-1)		31.67	31.03	28.81	29.10
(*sn*-2)			30.89	28.67	
δ				31.54	29.19
				31.48	
ϵ					31.86
$\omega - 1$	18.01	22.65	22.02	22.46	22.58
ω	12.85	13.52	13.46	13.73	13.79
carbonyl	176.46	174.2	174.21	174.09	173.95
	176.00	173.9			

The chemical shifts of the carbon resonances are given in Table I. The carbonyl assignment is straightforward and has been verified for the carbonyls of dihexanoyl-PC (Schmidt et al., 1977). The glycerol backbone and choline head group assignments are based on an earlier study of dipalmitoyl-PC (Birdsall et al., 1972) and have been confirmed by (i) comparison of ^{1}H irradiated and fully coupled spectra, (ii) virtually identical chemical shifts for these carbons in short-chain compounds and for long-chain lipids in CD_3OD or $CDCl_3$, and (iii) similarity of $J^{31}_{P-}{}^{13}_{C}$ and ^{14}N broadening of directly bonded carbons. Interestingly, there is little dependence of the chemical shift for the head group and glycerol backbone resonances on phosphatidylcholine structure (monomer, micelle, or bilayer).

Figure 1 shows the chemical shifts and assignments for the acyl chain region of the spectrum. Resonances for all positions are resolvable. The assignments of specific carbons are based on the Lindeman–Adams parameters for paraffin ^{13}C chemical shifts, with the acyl chain treated as the appropriate methyl ester (Klein & Kemp, 1977). Assignments for the γ and δ carbon atoms of dioctanoyl-PC are based on the effect of the carbonyl on the two resonances. Assignments for the α and β carbon atoms were checked by a selective ^{1}H irradiation decoupling experiment, using proton assignments determined previously (Roberts et al., 1978; Hershberg et al., 1976). For several carbon atoms in the acyl chains, the resonances are split. There are several possible causes for this: slow exchange of two micelle populations, slow exchange of fatty acyl conformations, or conformationally distinct *sn*-1 and *sn*-2 fatty acyl chains. In Table II we show the alkyl chain chemical shifts for 1-hexanoyl-2-perdeuteriohexanoyl-PC. Only one resonance is detected for each of the α-, β-, and γ-methylene carbons. The intensity of carbons on the *sn*-2 chain will be decreased dramatically because of coupling to ^{2}H, which is not collapsed by broad-band ^{1}H decoupling, and the lack of

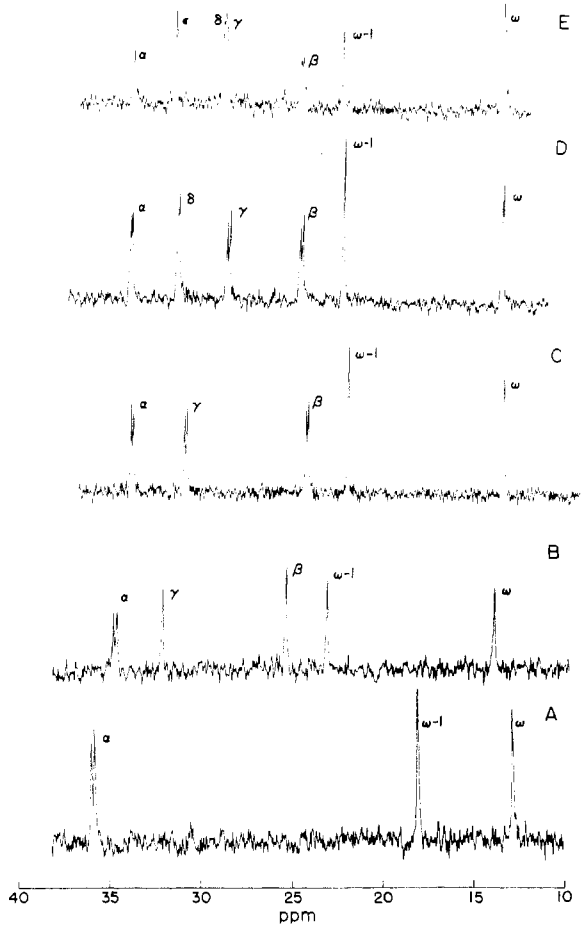

FIGURE 1: ^{13}C NMR spectra of the alkyl carbon region of short-chain phosphatidylcholines: monomers of (A) aqueous dibutyryl-PC and (B) dihexanoyl-PC in CD_3OD; micelles of (C) dihexanoyl-PC with 1.0 M NaCl added, (D) diheptanoyl-PC, and (E) dioctanoyl-PC with 0.2 M KSCN. Aqueous solutions contain 0.05 M sodium phosphate and 0.005 M EDTA, pD 7.9. Assignments of individual carbons are indicated.

Table II: ^{13}C NMR Chemical Shifts (Parts per Million from External Me_4Si) for Alkyl Carbons of 1-Hexanoyl-2-perdeuteriohexanoyl-PC with Added Dihexanoyl-PC

alkyl carbon	1-C_6H_{13}-2-C_6D_{13}-PC	1,2-C_6H_{13}-PC[a]
α (*sn*-2)		33.85
(*sn*-1)	33.74	33.74
β (*sn*-2)		24.34
(*sn*-1)	24.20	24.23
γ (*sn*-1)	30.89	30.90
(*sn*-2)		30.78
ω − 1	21.96	21.93
ω	13.44	13.43

[a] Absolute chemical shifts for the alkyl carbons depend on the phospholipid concentration (i.e., monomer/micelle ratio); to account for this variability we have reported δ_C for the deuterated lipid (19 mM) and then diluted the sample to 12.7 mM with the fully protonated lipid, dihexanoyl-PC (13.3 mM), for direct comparison of *sn*-1/*sn*-2 carbon atoms.

an appreciable NOE. The carbons from the *sn*-1 chain coincide with the upfield resonances of the α and β doublets and the downfield resonance of the γ-methylene carbon in dihexanoyl-PC. Thus, for the α and β carbons the lower field resonance and for the γ carbon the upfield resonance must arise from the *sn*-2 chain, and magnetic nonequivalence is the origin of the splitting.

Monomer phospholipids (dibutyryl-PC, dihexanoyl-PC in CD_3OD) show chain–chain differentiation for the α carbon only, while micellar lipids show magnetically nonequivalent carbons at α, β, γ, and sometimes δ positions. Thus, chain–chain differentiation as detected by ^{13}C chemical shifts is clearly dependent on the aggregation state of phosphatidylcholine. Other trends are evident: the chemical-shift differences for the α carbons decrease when the lipid goes from monomer to micelle, while the shift difference between β and γ carbons is only evident in the micellar species. For dihexanoyl-PC and diheptanoyl-PC, alkyl carbon line widths are in the range 5–8 Hz. A detailed analysis of the alkyl chemical shifts for the dihexanoyl-PC monomer to micelle transformation will be published elsewhere (R. A. Burns, Jr., and M. F. Roberts, unpublished experiments). Differentiation of the *sn*-1 and *sn*-2 chains is not as pronounced with dioctanoyl-PC methylenes. Splitting of the α carbons is not distinct, although the line broadening and peak height suggest that a 1–3 Hz difference still occurs. Only the β carbons of the two chains are well resolved; the γ and δ carbons overlap and make observation of *sn*-1/*sn*-2 differences difficult.

Relaxation Data. Table III summarizes the spin–lattice relaxation (T_1) data for each carbon of the short-chain phosphatidylcholines. Dihexanoyl-PC exists as a monomer in methanol, and serves as a control with dibutyryl-PC to analyze the contribution of micelle formation to the relaxation data.

Any interpretation of NMR relaxation parameters requires the establishment of the relaxation mechanism. Measurement of the nuclear Overhauser effect (NOE) can determine the contribution of C–H dipolar interactions to the relaxation (Noggle & Schirmer, 1971). We find average NOEs of 2.6 ± 0.2 for most carbon atoms in the short-chain lipids, either as monomer or micelles. An NOE of 2.99 would indicate relaxation exclusively by the dipolar interaction with neighboring protons. Only the carbonyl (average NOE = 1.8) and the glycerol C–H (average NOE = 1.9) deviate from the larger NOEs generally observed. NOEs are independent of micelle formation. There is an approximate twofold increase in T_1s observed at 328 K compared to values obtained at 303 K, indicating that extreme narrowing conditions apply. Under this limit a longer T_1 implies that faster motions dominate the carbon–proton dipolar interaction.

There is a two- to threefold difference in T_1 between monomer and micelle carbon atoms. As can be seen for the series dihexanoyl-PC, diheptanoyl-PC, and dioctanoyl-PC, the larger the micelle the shorter the T_1. From the experimental T_1s one can calculate an effective correlation time, τ_{eff}, assuming that dipolar interactions with N directly bonded protons dominate the relaxation:

$$\frac{1}{NT_1} = \frac{\gamma_c^2\gamma_H^2\hbar^2}{r^6}\left[\frac{3}{10}\frac{\tau_{eff}}{1+\omega_c^2\tau_{eff}^2} + \frac{1}{10}\frac{\tau_{eff}}{1+(\omega_C-\omega_H)^2\tau_{eff}^2} + \frac{3}{5}\frac{\tau_{eff}}{1+(\omega_C+\omega_H)^2\tau_{eff}^2}\right]$$

γ_C and γ_H are the gyromagnetic ratios for carbon and hydrogen, respectively, r is the C–H internuclear distance (1.095 Å), and $\omega_{C,H} = \gamma_{C,H} \times H_0$ (H_0 = 63.4 kG) (Noggle & Schirmer, 1971). Although this relation is strictly valid for isotropic motion, the τ_{eff} determined from it serves as a means of qualitatively assessing the same carbons in the different lecithin species. In Table IV are shown τ_{eff} values calculated for the glycerol backbone, choline methylene, and average (or plateau) alkyl methylene carbons. The glycerol backbone shows some sensitivity to the micelle size. The τ_{eff}s of the backbone carbons are always the longest found in the molecule.

Table III: ^{13}C NMR Spin-Lattice Relaxation Times for Monomer, Micellar, and Bilayer Phosphatidylcholines

	T_1 (s)					
	monomer		micelle			bilayer
carbon atom	dibutyryl-PC	dihexanoyl-PC (CD_3OD)	dihexanoyl-PC	diheptanoyl-PC	dioctanoyl-PC	dimyristoyl-PC[a]
backbone						
CHO	0.76 (0.15)[b]	0.79 (0.15)	0.36 (0.08)	0.20 (0.04)	0.22 (0.04)	0.15
CH_2OP	0.32 (0.04)	0.52 (0.03)	0.24 (0.02)	0.18 (0.08)	0.12 (0.09)	0.10
CH_2O	0.39 (0.01)	0.52 (0.02)	0.26 (0.04)	0.37 (0.11)	0.09 (0.05)	
head group						
CH_2N	0.82 (0.35)	0.86 (0.06)	0.51 (0.15)	0.48 (0.05)	0.49 (0.01)	0.40
CH_2OP	0.82 (0.10)	0.92 (0.10)	0.55 (0.04)	0.48 (0.07)	0.53 (0.05)	0.35
$\overset{+}{N}(CH_3)_3$	1.09 (0.03)	1.08 (0.10)	0.85 (0.02)	0.72 (0.03)	0.68 (0.04)	0.76
alkyl						
α (*sn*-2)	1.49 (0.07)	1.02 (0.16)	0.42 (0.03)	0.51 (0.03)	0.41 (0.08)	0.20
(*sn*-1)	1.40 (0.05)	1.01 (0.17)	0.61 (0.08)	0.67 (0.11)		
β (*sn*-2)		2.06 (0.35)	0.69 (0.03)	0.36 (0.02)	0.41 (0.04)	0.24
(*sn*-1)			0.89 (0.10)	0.50 (0.07)	0.45 (0.11)	
γ (*sn*-1)		2.90 (0.20)	0.90 (0.09)	0.64 (0.16)	0.60 (0.05)	0.48
(*sn*-2)			0.89 (0.08)	0.73 (0.17)		
δ				0.61 (0.13)	0.62 (0.03)	
				0.53 (0.16)		
ϵ					0.92 (0.10)	
$\omega - 1$	2.26 (0.12)	3.85 (0.23)	1.32 (0.28)	1.20 (0.04)	1.58 (0.07)	1.70
ω	3.34 (0.20)	4.29 (0.26)	2.68 (0.27)	2.22 (0.43)	1.87 (0.53)	3.80
carbonyl	5.14 (1.72)	5.13 (0.85)				
	6.09 (0.56)	6.03 (2.25)	2.96 (0.12)	2.24 (0.06)	2.39 (0.29)	

[a] T_1 values for sonicated vesicles of dimyristoyl-PC are from Lee et al. (1976). [b] The weight-averaged error in T_1, calculated as described under Experimental Procedure, is indicated in parentheses.

Table IV: Apparent Molecular Weights and Correlation Times for Short-Chain Phosphatidylcholines

phospholipid	mol wt	τ_c (ps)	$\tau_{eff,glycerol}$[b] (ps)	$\tau_{eff,choline}$[b] (ps)	$\tau_{eff,alkyl}$[b] (ps)
dibutyryl-PC	415.4		68	30	13
dihexanoyl-PC (CD_3OD)	471.6[c]		48	27	9
dihexanoyl-PC	15 000[d]	2.7×10^{-4}[a]	110	43	28
diheptanoyl-PC	78 000[d]		110	48	45
dioctanoyl-PC	4 300 000[e]		250	48	30

[a] τ_c is the micelle rotational correlation time calculated for dihexanoyl-PC according to the Stokes–Einstein equation. [b] τ_{eff} is the effective rotational correlation time derived from the ^{13}C NT_1 value. [c] Dihexanoyl-PC exists as a monomer or very small aggregate in methanol. [d] Tausk et al. (1974a). [e] Tausk et al. (1974b).

These two facts indicate relative rigidity in this part of the molecule, with a common motion dominating relaxation of all three backbone carbon atoms. τ_{eff} calculated for the choline methylene carbons shows significant sensitivity only to participation of the molecule in a micelle, even though micelle size varies by two orders of magnitude. This suggests motional characteristics of the head group which are dependent only on the existence of an interface. For dihexanoyl-PC, which is the closest of the micelles to a sphere (Tausk et al., 1974a–c), one can estimate an overall micelle rational correlation time based on the Stokes–Einstein equation. From Table IV it is quite obvious that for micellar dihexanoyl-PC the overall micelle correlation time is much longer than τ_{eff}; i.e., overall micelle rotation has little influence on the ^{13}C T_1s observed.

The acyl chains show the most interesting trend in relaxation times. Differences in the T_1 values between the *sn*-1 and *sn*-2 chains for the α and β carbons of micellar dihexanoyl-PC and for the β carbons of diheptanoyl-PC are considered experimentally significant. Application of the normal deviate test (Meyer, 1975) for the T_1 differences between *sn*-1 and *sn*-2 carbons in micellar dihexanoyl-PC shows that the probability that the difference is due to chance is 0.02, 0.05, and 0.94 for the α, β, and γ carbons, respectively. For micellar diheptanoyl-PC, these probabilities are 0.16, 0.05, 0.60, and 0.70 (α, β, γ, and δ). The slight difference in T_1s for the β carbons of dioctanoyl-PC is insignificant. For monomer PCs the α-methylene carbons and the carbonyls of the two chains have indistinguishable relaxation times. In each case, where there is a significant difference in T_1 (i.e., micellar α and β carbons) the *sn*-1 carbons have the longer T_1 values, further suggesting that the phenomenon is real.

Figures 2 and 3 demonstrate graphically the differences in T_1 observed along the alkyl chains. A linear increase in T_1 is observed at each position in the chain for monomeric lipids. The micellar species show different relaxation behavior. T_1 increases for α and β carbons, remains constant (plateau) throughout the center of the chain, and then increases for the $\omega - 1$ and ω carbons. The concentrations of lipids used in the micelle T_1 studies ensure that for dihexanoyl-PC less than 10% of the lipid is monomeric and in fast exchange with the micellar lipid (the presence of NaCl has substantially lowered the cmc); for diheptanoyl-PC and dioctanoyl-PC, monomeric lipids are 2–3 and 0.3–0.4%, respectively. For dihexanoyl-PC, the presence of monomer lipid tends to affect the plateau region of the T_1 profile; i.e., removing the monomer contribution to T_1 would flatten the micelle curve even more.

Discussion

Short-Chain Phosphatidylcholines and Other Lecithin Aggregates. A well-defined picture of a phospholipid molecule in bilayers has been generated by a number of physical studies (Seelig & Niederberger, 1974; Yeagle, 1978; Lee et al., 1976;

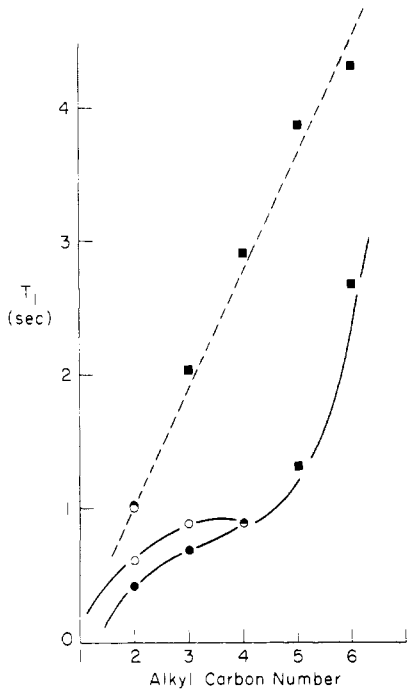

FIGURE 2: ^{13}C spin–lattice relaxation times of monomer dihexanoyl-PC in CD_3OD (---) and micellar dihexanoyl-PC (—) as a function of the carbon atom position of the alkyl chains: T_1 values for the *sn*-1 (○) and *sn*-2 (●) methylene carbons are shown where the two chains are magnetically nonequivalent; (■) values for carbon positions where the two chains appear equivalent.

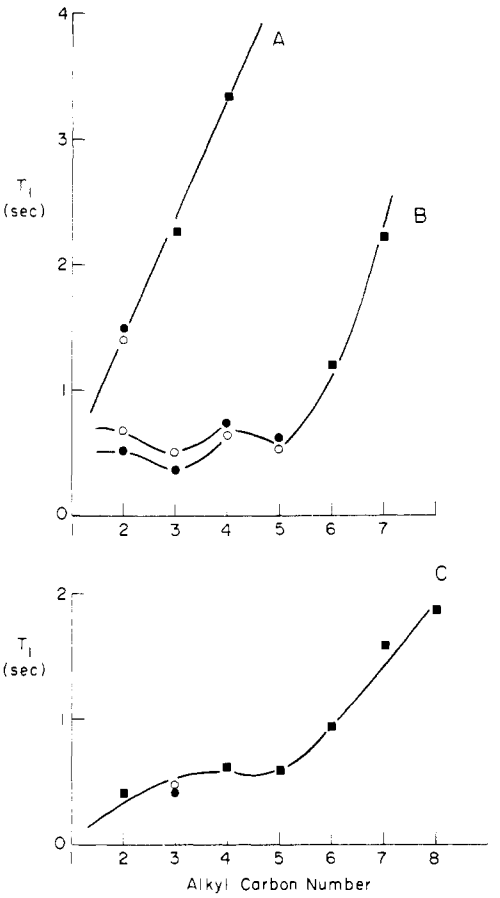

FIGURE 3: ^{13}C spin–lattice relaxation times of the fatty acyl carbons of monomer dibutyryl-PC (A), and micellar diheptanoyl-PC (B) and dioctanoyl-PC (C). When the two chains are magnetically nonequivalent, separate values are shown for the *sn*-1 (○) and *sn*-2 (●) carbons.

Buldt et al., 1978; Elder et al., 1977). The glycerol backbone is the most rigid section of the molecule, while the head group and fatty acyl chains are capable of increased motion. The phosphoester head group is aligned parallel to the bilayer plane. The two fatty acyl chains are conformationally nonequivalent: the beginning of the *sn*-2 chain is oriented parallel to the interface, while the *sn*-1 chain is perpendicular to the surface. This causes the *sn*-1 chain to extend about 1.5 carbon–carbon bonds beyond an equivalent length *sn*-2 chain. Fatty acyl chain nonequivalence appears to be a general feature of most phospholipids in bilayers (Seelig & Browning, 1978) and also occurs in phospholipid/detergent mixed micelles (Roberts et al., 1978).

The ^{13}C NMR data presented here indicate striking similarities between short-chain lecithin micelles and other lipid aggregates. Chemical shifts are similar to the corresponding resolvable peaks in sonicated vesicles (Birdsall et al., 1972) and liposomes (Metcalfe et al., 1971). Relative differences in spin–lattice relaxation times among the glycerol backbone, choline head group, and acyl chains are similar to those observed for long-chain lipids in sonicated vesicles (Lee et al., 1976) or detergent mixed micelles (Ribeiro & Dennis, 1976). In micelles, NT_1 is shortest for carbon atoms in the glycerol backbone, consistent with bilayer studies. Relaxation times for choline methylene and *N*-methyl carbon atoms are virtually identical for micellar dihexanoyl-PC, diheptanoyl-PC, and dioctanoyl-PC. Thus, common motions which are not affected by micelle size dominate the head groups in lecithin aggregates. For bilayers these motions include rotation of the choline around an axis perpendicular to the bilayer surface.

The main difference between the ^{13}C NMR parameters of the lecithin micelles and bilayers is the clear observation of fatty acyl chain nonequivalence in the micellar system. The chemical-shift difference for a given carbon atom in the two chains (4–12 Hz) is easily detected because of narrow line widths (5–8 Hz) for the micellar alkyl carbons. This chain–chain differentiation opens up the possibility of examining the effect of metal ions, cholesterol, peptides, etc., on the two acyl chains separately. No other simple nonenriched phospholipid system offers this potential.

Methylene carbon chemical shifts for an alkyl chain have been related to the population of trans/gauche isomerizations (Batchelor et al., 1972). From these arguments egg lecithin fatty acyl chains have on the average a more trans orientation in sonicated vesicles than in $CHCl_3$, where monomers or inverted micelles are formed; hence the methylene carbons are shifted downfield. If we assume that this behavior holds for the alkyl carbons in short-chain phosphatidylcholine micelles, on the average the *sn*-2 chain has a slightly more trans conformation than the *sn*-1 chain.

Although the *sn*-1 and *sn*-2 chains are magnetically distinguishable as far as four carbons from the carbonyl, differences in spin–lattice relaxation times for the two chains only occur for the first two carbons. If rotational diffusion of the molecule in the interfacial plane contributes to the relaxation, and comparable *sn*-1 and *sn*-2 carbons lie at different distances from the molecular axis, then a different contribution to relaxation for the α and β carbons is likely. The indistinguishable T_1s found for the γ and δ carbons are compatible with the interpretation of similar studies in several other model systems (Wittebort and Szabo, 1978). If the rotational diffusion rates about carbon–carbon bonds in a chain are greater than the molecular diffusion rate, the contribution of molecular motion to T_1 relaxation is eliminated several atoms down the chain

Table V: Comparison of ^{13}C T_1 Values Calculated from ^{2}H NMR Relaxation Parameters with Observed Values for Micellar Phosphatidylcholine

carbon atom	τ_c[a]	$T_{1(calcd)}$ (s)	$T_{1(obsd)}$ (s) dihexanoyl-PC	diheptanoyl-PC	dioctanoyl-PC
α	8×10^{-11}	0.3	0.5[b]	0.3[b]	0.4
β, γ, δ, etc.	6×10^{-11}	0.4	0.8[b]	0.6[b]	0.6
$\omega - 1$	1×10^{-11}	2.4	1.3	1.2	1.6

[a] Brown et al. (1979); values of τ_c for dipalmitoyl-PC (80 °C) considerably above its phase transition temperature are used. [b] Average value of *sn*-1 and *sn*-2 methylene carbon T_1 values.

by the faster motion about the intervening chemical bonds.

Comparison of ^{2}H and ^{13}C Relaxation Profiles: Implications for Micelle Structure. The change in ^{13}C T_1 observed along the acyl chain is quite similar to relaxation parameters found in ^{2}H NMR studies of dipalmitoyl-PC liposomes (Brown et al., 1979). Deuterium T_1 relaxation is dominated by a different relaxation mechanism than ^{13}C: the quadrupolar interaction. The correlation time modulating this interaction is the rotational reorientation of the carbon–deuteron bond. Deuterium T_1 studies thus focus on motion at the carbon center under investigation. Correlation times calculated from the ^{2}H relaxation studies predict the ^{13}C dipolar relaxation astonishingly well (Table V). (The terminal methyl carbon is the only one with an observed T_1 much shorter than that predicted from ^{2}H NMR. This may reflect decreased motion caused by the tighter packing in the micelle center compared to a bilayer. Alternatively, increasing the number of methylene groups may add to the rotational motion of the terminal methyl carbon in long-chain phospholipids.) Thus, local motion of the carbon–hydrogen bond seems to dominate ^{13}C relaxation as well. The comparison between the bilayer and micelle suggests that motions in the acyl chains are the same.

Monomer/Micelle Changes and Interfacial Activation. A characteristic of many soluble lipolytic enzymes is their "activation" by interfaces. Given the choice of a monomeric phospholipid or one incorporated into an interface, many lipases prefer the interfacial substrate (Verger & de Haas, 1976). This phenomenon has been examined in detail using the short-chain phosphatidylcholines. Below their cmc's these lipids are poor substrates for phospholipase A_2 and phospholipase C; above the cmc's where phospholipid is micellar, the enzyme activities increase by factors of 20–100.

Two major hypotheses have been proposed to explain interfacial activation: (1) the soluble enzyme undergoes a conformational change upon interacting with the interface; and (2) the phospholipid molecule is altered to a form that is a more efficient substrate (conformational change, dehydration, etc.) when introduced into an interfacial matrix. Although enzyme conformational changes occur upon binding interfacial phospholipid, none has been shown to be responsible for the rate enhancement. Direct evidence for a change in phospholipid structure is limited to two NMR studies. ^{1}H NMR studies of short-chain phosphatidylcholines as monomers and intercalated into detergent micelles have shown that the transformation of phospholipid monomer to micelle accentuates the difference in the two fatty acyl chains, placing the *sn*-1 chain in the more hydrophobic environment (Roberts et al., 1978). ^{13}C NMR studies of enriched carbonyls in dihexanoyl-PC suggest that the extent of hydration is the major difference between monomer and micelle (Schmidt et al., 1977). Indirect evidence is that the presence of micellar dioctanoyl-PC does not accelerate the phospholipase A_2 catalyzed hydrolysis of monomeric dibutyryl-PC (Wells, 1974).

Our ^{13}C NMR studies show that changes in the phospholipid molecule indeed occur upon aggregation, although the geometric details of the changes are not known. Major changes are sensed in the alkyl region where chain–chain differentiation is dramatically enhanced upon micellization. These changes can be interpreted in two ways. (1) There is a conformational change in the phospholipid such that the two chains are in different orientations in micelles compared to monomer. (2) Monomer and micellar lipid have the same basic conformation; the difference lies in carbonyl and head-group dehydration and motional restriction (trans/gauche isomerizations) of the two chains for interfacial lipid. Either may have a profound effect on phospholipase activity. ^{1}H NMR analysis of the coupling pattern of glycerol protons in dibutyryl-PC shows no pronounced differences from micelles of dihexanoyl-PC or diheptanoyl-PC (M. F. Roberts, unpublished results), arguing for similar conformations for the backbone. Since there is virtually no change in chemical shifts and only a small change in T_1s for the phosphocholine carbons upon micellization, major conformational changes probably do not occur. Further studies are in progress to define the alkyl ^{13}C chemical shifts in terms of conformational changes and solvent effects. Thus, ^{13}C NMR studies offer the potential for correlation of micelle-induced changes in the lecithin with phospholipase interfacial activation.

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Activation of Histamine Secretion from Rat Mast Cells by Aqueous Dispersions of Phosphatidylserine†

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ABSTRACT: Aqueous dispersions were generated from bovine brain phosphatidylserine (PS) and three synthetic derivatives: 1,2-dimyristoyl-, 1,2-dipalmitoyl-, and 1,2-distearoyl-*sn*-3-glycerophosphoserine (DSPS). The gel–liquid-crystalline phase transition temperatures of the dispersions were determined with a fluorescence probe and by 90° light-scattering measurements. The effectiveness of the dispersions in activating histamine secretion from mast cells stimulated with concanavalin A was examined, and the results were evaluated in terms of the differences in acyl chain composition and phase transition temperature. At 22 °C, fluid-phase bovine brain PS dispersions were more active on a molecular basis than solid-phase DSPS dispersions, whereas at 37 °C, the solid-phase DSPS dispersions were more active. When the relative activities of three different solid-phase PS dispersions were compared, activity was found to be dependent on the length of the acyl chains of the PS molecule with longer chains being more effective. Since the binding isotherms for the interaction of all three solid-phase PS dispersions with mast cells were virtually indistinguishable, the greater activity of PS molecules with longer acyl chains could not be explained on the basis of an enhanced binding capability. The mechanism by which PS vesicles interact with mast cells was further studied by trapping the hydrophilic fluorescent dye carboxyfluorescein within the aqueous compartment of the vesicles. Incubation of such dye-loaded vesicles with cells resulted in considerable release of the dye from the vesicles into the extracellular medium with little transfer of dye from the vesicles to the cells. The rate of extracellular dye release was saturable in the concentration range over which the vesicles were found to activate secretion. PS vesicles but not phosphatidylcholine vesicles afforded competitive inhibition of extracellular dye release. On the basis of these results and previous observations on the specificity and selectivity of the effect of PS on mast cell secretion, a tentative model is presented to explain the mechanism of PS action.

The mast cell is a highly specialized secretory cell ubiquitous in the connective tissue of man and other vertebrates. Although a detailed understanding of the function of the mast cell is lacking, the demonstration of histamine and other inflammatory mediators within its secretory granules and their release upon immunologic challenge (Becker & Henson, 1973) serve as the basis for association of this cell with the pathophysiology of immediate hypersensitivity and anaphylaxis (Lewis & Austen, 1977). In conjunction with its immunopathologic relevance, the mechanism of histamine secretion from mast cells has been the focus of numerous experimental investigations as a system for correlative study of the bio-

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