Polar Group Conformation of Phosphatidylcholine. Effect of Solvent and Aggregation†

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ABSTRACT: From ^{1}H and ^{13}C high-resolution NMR spectra of phosphatidylcholines and a lysophosphatidylcholine analogue, 1-lauroylpropanediol-3-phosphorylcholine (LPPC), vicinal ^{1}H–^{1}H, ^{14}N–^{1}H and ^{31}P-^{13}C spin coupling constants were derived. These coupling constants were used to calculate rotamer populations for the C–C and C–O bonds of the polar group of the above lipids by using a Karplus treatment. There is a preferred conformation in the phosphorylcholine fragment which is similar for all compounds investigated under various experimental conditions. This preferred conformation is characterized by an almost exclusively gauche conformation of the choline group (torsion angle α_5) and predominantly antiperiplanar conformations about the C–C–O–P and P–O–C–C bonds. There is a significant difference in the conformation between the glycerol group of diacylphosphatidylcholines and the propanediol group of LPPC. While there is a well-defined conformation in the glycerol group of diacylphosphatidylcholine, no conformational preference about the two C–C bonds of 1,3-propanediol is observed in LPPC. In diacylphosphatidylcholine the conformations favored about the C1–C2–C3–O31 (glycerol) bond (torsion angles θ_3 and θ_4) are those which allow the parallel alignment of the hydrocarbon chain, thus optimizing hydrophobic interactions. This is true in both D_2O and organic solvent and for dihexanoylphosphatidylcholine at concentrations below and above the cmc. Apparently chain stacking is energetically favored also in the monomeric form. The molecule is flexible about the second glycerol C–C bond (O11–C1–C2–C3; torsion angles θ_1 and θ_3) which determines the orientation of the phosphorylcholine group relative to the two hydrocarbon chains. Within the limitations of the spin coupling analysis, the preferred conformation of the phosphorylcholine fragment of lipids in solution is in good agreement with the crystal structure of LPPC except that α_4 is closer to antiplanar in solution while it is anticlinal in the crystal. The preferred conformation of the phosphorylcholine group is independent of the number of acyl chains, of solvation, and of the state of aggregation. Replacement of water of hydration by CD_3OD or CD_3OD–$CDCl_3$ had little or no effect on the polar group conformation. There is no significant difference in the preferred conformation of the polar group of dihexanoylphosphatidylcholine in the monomeric and the micellar aggregated form, suggesting that the conformation is primarily determined by the intramolecular energetics of the compound. By comparison with long-chain phosphatidylcholines present in hydrated bilayers [Seelig, J., Gally, H.-U., & Wohlgemuth, R. (1977) *Biochim. Biophys. Acta 467*, 109–119], it is concluded that the conformation of the phosphatidylcholine polar group is the same in the monomeric, micellar, and bilayer state.

Phospholipid bilayers are integral parts of biological membranes. The knowledge of the molecular structure of phospholipids is therefore essential for an understanding of their functional role in biological membranes. In this paper the conformation of the polar group of phosphatidylcholine, one of the major phospholipids of cell and subcellular membranes, is investigated under various experimental conditions by using high-resolution NMR techniques. By changing the solvent the effect of solvation and by working below and above the cmc the effect of aggregation on the conformation of the polar group (glycerylphosphorylcholine) are examined. Conformational information is derived from vicinal H–H, H–C, and H–P coupling constants. The principle underlying this method is that vicinal spin coupling constants depend on the torsion angle.

Phospholipids in aqueous dispersions and dissolved in organic solvents have a great deal of molecular and segmental motion at room temperature, and, consequently, motionally averaged conformations are observed. The NMR results obtained with these disordered, liquid-crystalline systems will be compared with the single crystal structure of a lysophosphatidylcholine analogue, 1-lauroylpropanediol-3-phosphorylcholine. Such a comparison should give an answer to the important question of whether the unique conformation present in the crystal is also present in solution or whether significant populations of other conformations exist. Furthermore, the comparison of the conformations (present in solution) in the monomeric and aggregated state of the lipid should shed light on the question of what kind of role intra- and intermolecular forces play in determining the polar group conformation.

Experimental Section

Materials

Dipalmitoylphosphatidylcholine (DPPC)[1] was synthesized as described (Paltauf et al., 1971). 1-Lauroylpropanediol-3-phosphorylcholine (LPPC) was synthesized from 1-lauroyl-1,3-propanediol and (β-bromoethyl)phosphoryl dichloride in the presence of excess triethylamine according to the method of Hirt & Berchtold (1958). Crystals of the monohydrate were obtained by controlled cooling of hot dichloromethane solutions. Anal. Calcd for $C_{20}H_{42}NO_6P{\cdot}H_2O$ (M_r 441.54): C, 54.4; H, 10.0; N, 3.17; P, 7.02. Found: C, 54.0; H, 10.3; N,

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[1] Abbreviations used: DHPC, 1,2-dihexanoyl-3-*sn*-phosphatidylcholine; DPPC, DL-dipalmitoylphosphatidylcholine; LPPC, 1-lauroylpropanediol-3-phosphorylcholine; cmc, critical micellar concentration; TSS, sodium 3-(trimethylsilyl)propanesulfonate.

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Table I: Chemical Shifts of the Polar Group Signals of Diacylphosphatidylcholines and 1-Lauroylpropanediol-3-phosphorylcholine

signals		chemical shifts (ppm)[a] DHPC in D_2O below cmc (6.4 mM)	DHPC in D_2O above cmc (95.7 mM)	DHPC in CD_3OD	DPPC in $CDCl_3$-CD_3OD		LPPC in D_2O	LPPC in CD_3OD
CH_2-O-CO	H_A:	4.28	4.29	4.17	4.16		4.17	4.18
	H_B:	4.43	4.47	4.42	4.42			
CH-O-CO or CH_2[b]	H_C:	5.32	5.34	5.23	5.24	$H_C = H_D$:	1.96	1.94
CH_2-O-P	H_D:	4.05	4.07	4.00	4.00	$H_E = H_F$:	3.94	3.95
	H_E:	4.06	4.08	4.01	4.00			
O-P-CH_2	$H_X = H_{X'}$:	4.30	4.33	4.28	4.25		4.27	4.25
CH_2N	$H_M = H_{M'}$:	3.66	3.71	3.64	3.60		3.67	3.62
$N(CH_3)_3$		3.22	3.27	3.22	3.23		3.23	3.21

[a] Chemical shifts are expressed in ppm downfield from TSS and tetramethylsilane used as internal references in D_2O and organic solvents, respectively. The accuracy of the chemical shifts is ±0.01 ppm. [b] It is the CH-OCO group for the diacylphosphatidylcholines and the CH_2 group in the 2 position of the propanediol group for the lysophosphatidylcholine analogue. The two CH_2 protons of that group are both chemically and magnetically equivalent, at least to a first approximation (cf. Table III).

3.10; P, 6.90. The crystallographic data and details of the single crystal structure are presented elsewhere (Hauser et al., 1980). Dihexanoylphosphatidylcholine (DHPC) was purchased from Applied Science Labs (State College, PA) and had a nominal purity of better than 99%. The purity of the lipids was monitored by TLC as described before (Hauser, 1971).

Methods

(*a*) *Determination of the Critical Micellar Concentration (cmc).* The cmc of DHPC and LPPC in H_2O was determined by ultrafiltration using Amicon ultrafiltration equipment with UM2 filters. The cmc was derived from the kink in the double-logarithmic plot relating the total lipid concentration to the lipid concentration in the filtrate. The values were 15.2 ± 1 mM, in good agreement with published work (de Haas et al., 1971; Tausk et al., 1974), and 0.33 ± 0.02 mM for DHPC and LPPC, respectively. The cmc of DHPC was determined independently from the distribution of rhodamine B between the aqueous phase and the micelle. The cmc derived from changes in fluorescence intensity when the dye was incorporated in the hydrophobic interior of the micelle was in good agreement within the error of the measurement with the value derived from ultrafiltration.

As reported before (Hershberg et al., 1976; Hauser et al., 1978a; Roberts et al., 1978), there are pronounced changes in the ^1H signals from the hydrocarbon chains when the DHPC concentration is raised above the cmc. The most prominent changes are observed in the terminal CH_3 and the α-methylene[2] signals, and it is convenient to use these resonances as a diagnostic means of deciding whether DHPC is present as monomers or micelles.

(*b*) *NMR Methods.* ^1H NMR spectra were recorded on a Bruker HXS-360 Fourier transform spectrometer with a digital resolution of 0.18 Hz/point. ^{13}C and ^{31}P NMR spectra were obtained at 100.6 MHz on a Bruker WH-400 and at 36.4 MHz on a Bruker HXE-90 spectrometer, respectively, both instruments operating in the Fourier transform mode. The chemical shifts and coupling constants of the resonances from the polar group were obtained from computer simulations of the spectra using the Nicolet ITRCAL version of the LAOCN3 program on a Nicolet B-NC12 computer equipped with a NIC-294 disk memory. Spin coupling constants were derived from the simulated spectra with an accuracy of about ±0.2 Hz. This error in the spin coupling constants will give an error in the major fractional populations of less than ±5%.

[2] Protons on the C atom next to the carbonyl group.

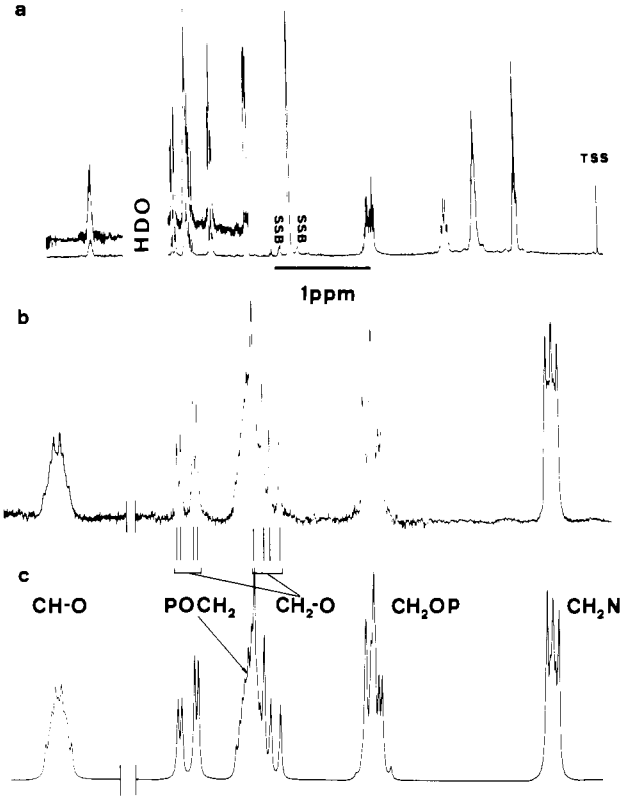

FIGURE 1: (a) 360-MHz ^1H NMR spectrum of dihexanoylphosphatidylcholine (~10 mg/mL = 21.2 mM) in D_2O at a nominal pH of 6 and at 25 °C; (b) the expanded spectrum of the lipid polar group except for the $N(CH_3)_3$ signal and (c) its computer simulation. SSB = spinning side band; in (b) and (c) the signal at the left arising from the single glycerol proton is not at its correct chemical shift.

Results

The ^1H NMR spectra of 1,2-dihexanoyl-*sn*-phosphatidylcholine (DHPC) in D_2O and in CD_3OD are given in Figures 1a and 2a, respectively. Figure 3a shows the ^1H NMR spectrum of DL-dipalmitoylphosphatidylcholine in $CDCl_3$–CD_3OD (2:1 v/v) and Figures 4a and 5a show the ^1H NMR spectra of a lysophosphatidylcholine analogue, 1-lauroylpropanediol-3-phosphorylcholine (LPPC) in D_2O and CD_3OD, respectively. The spectra in Figures 1b–5b are expansions of the polar group region, and computer simulations of the same

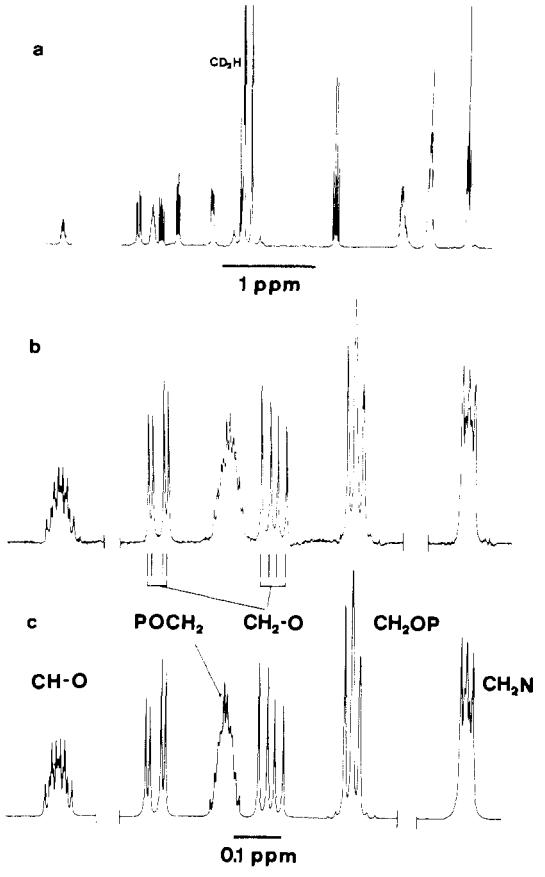

FIGURE 2: (a) 360-MHz ^1H NMR spectrum of dihexanoylphosphatidylcholine (~10 mg/mL = 21.2 mM) in CD_3OD at 25 °C; (b) the expanded spectrum of the lipid polar group except for the $N(CH_3)_3$ signal and (c) its computer simulation. The CH–OCO glycerol and the CH_2N signals in (b) and (c) are not at the correct chemical shifts.

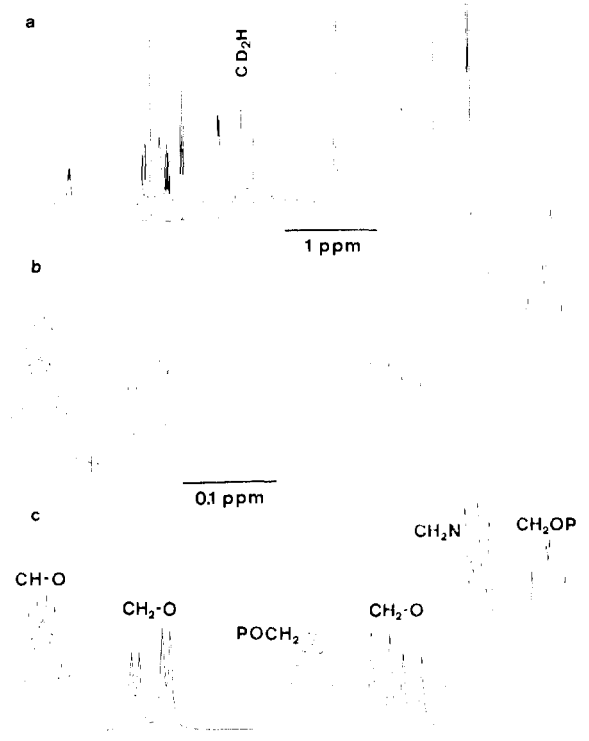

FIGURE 3: (a) 360-MHz ^1H NMR spectrum of DL-dipalmitoylphosphatidylcholine (27.5 mg/mL = 36.6 mM) in $CDCl_3$–CD_3OD (2:1 v/v) at 27 °C; (b) the expanded spectrum of the lipid polar group except for the $N(CH_3)_3$ singlet and (c) its computer simulation. The CH–OCO glycerol and the CH_2N signals in (b) and (c) are not at the correct chemical shifts.

spectral regions are presented in Figures 1c–5c. The assignment of the lipid polar group signals (Figures 1b–5b) summarized in Table I is based upon intensity measurements, homo- and heteronuclear double-resonance experiments, and changes in chemical shifts induced by shift and broadening probes of the lanthanide series. The values of the chemical shifts (Table I) are in good agreement with data in the literature (Finer et al., 1972a; Birdsall et al., 1972; Hauser et al., 1975; Hershberg et al., 1976; Roberts et al., 1978). While the chemical shifts of DHPC in CD_3OD were independent of concentration between 1 and 100 mM, there were significant spectral changes in both the polar group and the hydrocarbon chain region of DHPC in H_2O at the cmc. Increasing the concentration above the cmc induced a slight but significant downfield shift of the three choline signals whereas the changes in chemical shifts of the glycerol signals were almost within the error of the measurement. The chemical shifts of the four, clearly discernible hydrocarbon chain signals of the phospholipid spectra (Figures 1a–5a) are summarized in Table II. From this and from a comparison of Figures 1a and 2a, the spectral changes in the aliphatic region of DHPC in H_2O at the cmc are evident. The terminal CH_3 signal changed from a single triplet below the cmc to two partially overlapping triplets above the cmc (Table II; cf. Figures 1a and 2a). The quartet of intensity ratio 1:3:3:1 (not shown) arising from the α-methylene protons consisted of two partially overlapping triplets. A very similar quartet is observed for the α-methylene protons of DHPC in CD_3OD (Figure 2a and Table II). Above the cmc this simple quartet changes to a high-field triplet at 2.36 ppm from TSS (Table II) arising from the α-methylene

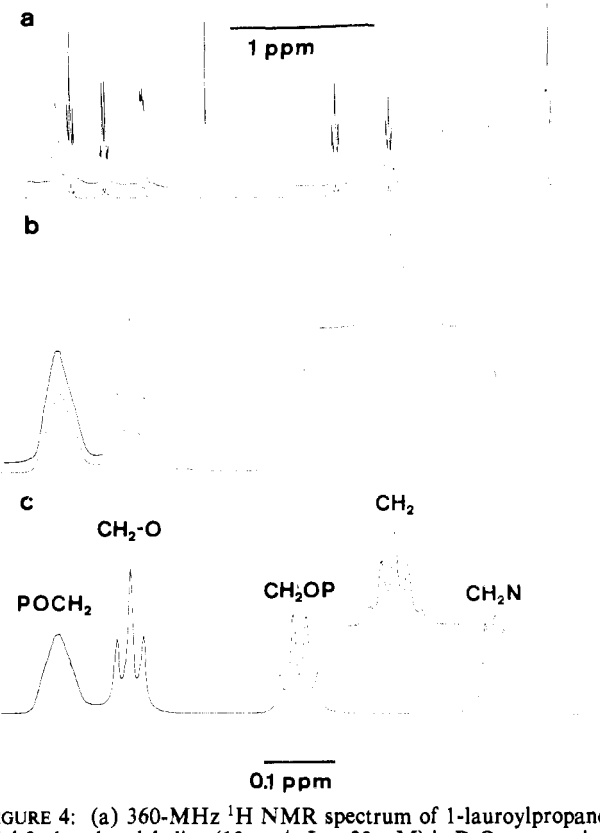

FIGURE 4: (a) 360-MHz ^1H NMR spectrum of 1-lauroylpropanediol-3-phosphorylcholine (13 mg/mL = 30 mM) in D_2O at a nominal pH of 6 at 25 °C; (b) the expanded spectrum of the lipid polar group except for the $N(CH_3)_3$ signal and (c) its computer simulation. In spectra (b) and (c) the signal from the CH_2 group (in the 2 position of the propanediol group) is inserted.

Table II: Chemical Shifts of the Hydrocarbon Chain Signals of Diacylphosphatidylcholines and of a Lysophosphatidylcholine Analogue

signal	chemical shifts (ppm)[a]					
	DHPC in D_2O below cmc (6.4 mM)	DHPC in D_2O above cmc (95.7 mM)	DHPC in CD_3OD	DPPC in $CDCl_3$-CD_3OD (2:1 v/v)	LPPC in D_2O	LPPC in CD_3OD
terminal CH_3	0.870	0.883; 0.890[b]	0.912; 0.915[b]	0.890	0.850	0.885
$(CH_2)_n$	1.30	1.33	1.33	1.27	1.26	1.28
CH_2-C-CO	1.61	1.63	1.61	1.61	1.58	1.59
CH_2-CO[c]	2.40; 2.42	2.37; 2.43; 2.40	2.32; 2.34	2.31; 2.33	2.32	2.31

[a] Chemical shifts are in ppm downfield from TSS and tetramethylsilane used as internal references in D_2O and organic solvents, respectively. The accuracy of the chemical shifts is ±0.005 ppm except for the terminal CH_3 signal which is accurate to ±0.4 Hz (0.001 ppm). [b] Chemical shifts of the two partially overlapping triplets. [c] Each of the two CH_2CO groups gives a triplet which have different chemical shifts such that a quartet results. This is true for diacylphosphatidylcholines dissolved in organic solvent and DHPC in D_2O at concentrations less than the cmc. The chemical shifts of the two triplets are given. The α-methylene protons of DHPC in D_2O at concentrations greater than the cmc give rise to a complex multiplet consisting of a triplet at 2.37 ppm (from the α-methylene protons of chain 1 (*sn*-1 position) and the AB part of an ABX_2 system). The chemical shifts of the AB protons are 2.40 and 2.43 ppm, respectively (Hauser et al., 1978; Roberts et al., 1978).

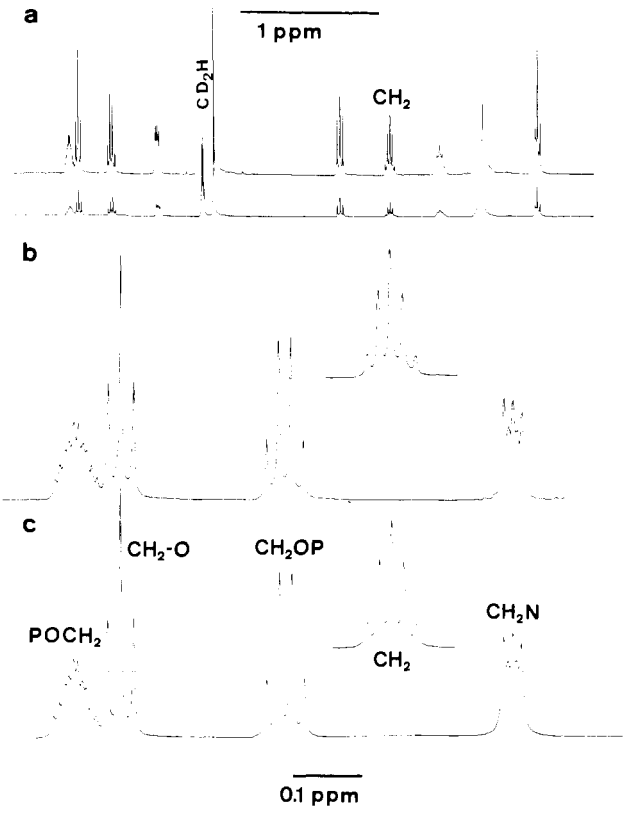

FIGURE 5: (a) 360-MHz ^1H NMR spectrum of 1-lauroylpropanediol-3-phosphorylcholine (20 mg/mL = 45.4 mM) in CD_3OD at 25 °C; (b) the expanded spectrum of the lipid polar group except for the $N(CH_3)_3$ signal and (c) its computer simulation. In spectra (b) and (c) the signal from the CH_2 group (in the 2 position of the propanediol group) is inserted.

protons of chain 1 (*sn*-1 position) and a low-field AB system of an ABX_2 pattern arising from the α-methylene protons of chain 2 (*sn*-2 position; Hauser et al., 1978a). The two nonequivalent α-methylene protons resonate at 2.40 and 2.43 ppm from TSS (Table II).

The spin coupling constants for the polar group signals derived from the simulated spectra (Figures 1c–5c) are summarized in Table III. The basic spectral parameters used as a starting point for the computation were either derived from the first-order analysis of the spectra or from spin decoupling experiments. From an inspection of the chemical shifts and coupling constants summarized in Tables I and III, it is clear that the spectra of the lysophosphatidylcholine analogue (Figures 4 and 5) are first-order spectra to a first approximation. In contrast, a first-order analysis is not applicable to the spectra of the diacylphosphatidylcholines (Figures 1–3). The most striking difference between the two types of spectra is the appearance of the CH_2OCO signal. With all diacyl compounds, this group gives an eight-line multiplet corresponding to the AB part of an ABX system. The two CH_2-OCO protons are therefore both chemically and magnetically nonequivalent. This is contrasted by the appearance of a simple triplet from the CH_2OCO group of LPPC (Figures 4 and 5), indicating that in this case the two CH_2OCO protons are both chemically and magnetically equivalent. In all compounds investigated the CH_2OCO group does not show any ^{31}P spin coupling. Irradiation of the CH–OCO group reduced the CH_2OCO octet to the four-line spectrum of the AB part, whereas irradiation of the CH_2 group of LPPC at 1.96 ppm (Figure 4) and at 1.94 ppm (Figure 5), respectively, reduced the CH_2OCO triplets to singlets. The nonequivalence of the CH_2OP glycerol protons in the DHPC spectra (Figures 1 and 2; cf. Tables I and III) is less pronounced (compared to the CH_2OCO signal); the CH_2OP glycerol groups of DPPC and LPPC (Figures 3–5) give first-order spectra to a first approximation, indicating that the protons are chemically and magnetically equivalent. The CH_2OP signals of all compounds show ^{31}P–^1H spin coupling; with LPPC the coupling constants can be readily derived from the doublet to which the CH_2OP signals collapsed when the neighboring protons were irradiated. With DHPC (Figures 1 and 2) the ^{31}P–^1H spin coupling was obtained from the ABX system to which the CH_2OP signal was reduced when the CH–OCO glycerol proton was decoupled.

In contrast to the spectral differences between the diacyl compounds and LPPC described above, the spectral region due to the choline group is very similar for all compounds investigated (Figures 1b,c–5b,c). The CH_2N group is always a quintet which is the MM′ part of an MM′XX′ system. With all compounds, irradiation of the $POCH_2$ (choline) signal caused the CH_2N quintet to collapse to a singlet. The $POCH_2$ (choline) multiplet is the asymmetric XX′ counterpart to the MM′ spin system. Its asymmetry arises from spin coupling of the $POCH_2$ protons to both ^{31}P and ^{14}N. This can be readily demonstrated by decoupling the CH_2N protons: in organic solvent the $POCH_2$ signal collapsed to an incompletely resolved sextet consisting of two partially overlapping 1:1:1 triplets which arise from coupling of the $POCH_2$ protons to the ^{14}N nucleus (spin 1). In contrast, irradiation of the CH_2N protons of DHPC and LPPC in D_2O yielded broad doublets, computer simulations of which showed that the ^{14}N–^1H spin coupling constants in D_2O are smaller than in organic solvents (cf. Table III). Furthermore, due to the small values of the ^{14}N–^1H spin coupling constants and due to the larger width of the lines

Table III: Coupling Constants of the Polar Group Resonances of Diacylphosphatidylcholines and of a Lysophosphatidylcholine Analogue

		coupling constants J (Hz)						
signal		DHPC in D_2O below cmc (6.4 mM)	DHPC in D_2O above cmc (21.2 mM)	DHPC in CD_3OD	DPPC in $CDCl_3$–CD_3OD		LPPC in D_2O	LPPC in CD_3OD
CH_2-O-CO	$^2J_{AB}$:	12.0	12.2	12.0	12.0		–[b]	–
	$^3J_{AC}$:	6.81	7.50	6.72	6.86	$^3J_{AC}$:	6.61	6.47
	$^3J_{BC}$:	2.96	2.70	3.32	3.09	$^3J_{BC}$:	6.73	6.47
CH-O-CO[a]	$^3J_{CD}$:	5.60	5.60	5.38	5.44	$^3J_{CE}$:	5.81	6.33
	$^3J_{CE}$:	3.80	3.80	5.19	5.54	$^3J_{CF}$:	6.01	6.33
	$^2J_{ED}$:	6.80	6.80	12.0	–		–	–
CH_2-O-P	$^3J_{PH_D}$:	7.10	7.10	6.10	7.00	$^3J_{PH_E}$:	7.02	6.75
	$^3J_{PH_E}$:	5.90	5.90	6.20	7.00	$^3J_{PH_F}$:	7.02	6.75
O-P-CH_2	$^2J_{XX'}$:	12.0	12.0	12.0	16.0		14.0	14.0
	$^3J_{PH_X} = {}^3J_{PH_{X'}}$:	6.50	6.50	6.70	7.54		6.00	6.50
	$^3J_{NH_X} = {}^3J_{NH_{X'}}$:	1.5–1.7	1.5–1.7	2.80	2.86		1.5–1.7	2.65
CH_2-N	$^2J_{MM'}$:	12.0	12.0	12.0	16.0		14.0	14.0
	$^3J_{MX} = {}^3J_{M'X'}$:	2.50	2.50	2.50	2.44		2.40	2.40
	$^3J_{M'X} = {}^3J_{MX'}$:	6.70	6.70	6.80	6.82		6.90	6.90

[a] It is the CH-OCO group for the diacylphosphatidylcholines and the CH_2 group in the 2 position of the propanediol group for the lysophosphatidylcholine analogue. [b] Due to the equivalence of the CH_2 protons, the geminal coupling constants $^2J_{AB}$, $^2J_{CD}$, and $^2J_{EF}$ are not observed.

Table IV: Minimum Free Energy Conformations and Rotamer Populations of the Polar Group of Diacylphosphatidylcholines[a]

			fractional population		
bond	torsion angle[b]	staggered conformations	DHPC in CD_3OD	DHPC in D_2O[c]	DPPC in $CDCl_3$–CD_3OD (2:1 v/v)
R_1COCH_2-$CHOCR_2$	θ_3 (θ_4)	gauche (–gauche)	0.40	0.33	0.41
		antiplanar (gauche)	0.50	0.60	0.52
		–gauche (antiplanar)	0.10	0.03	0.07
R_2COCH-CH_2OP	θ_1 (θ_2)	–gauche (gauche)	0.42 (0.42)	0.48 (0.53)	0.39 (0.39)
		antiplanar (–gauche)	0.29 (0.27)	0.37 (0.13)	0.28 (0.28)
		gauche (antiplanar)	0.29 (0.31)	0.15 (0.34)	0.33 (0.33)
$CHCH_2$-OP	α_1	±gauche	0.23	0.26	0.32
		antiplanar	0.77	0.74	0.68
PO-CH_2CH_2	α_4	±gauche	0.28	0.26	0.37
		antiplanar	0.72	0.74	0.68
$POCH_2$-CH_2N	α_5	±gauche	0.99	0.98	1.00
		antiplanar	0.01	0.02	0.00

[a] For torsion angles θ_1 and θ_2 two sets of values were obtained with $J_{CD} > J_{CE}$ and $J_{CD} < J_{CE}$ (in parentheses). For torsion angles θ_3 and θ_4 the set of values presented is obtained for $J_{AC} > J_{BC}$. The alternative with $J_{AC} < J_{BC}$ can be ruled out because in this case the rotamer with θ_4 = antiplanar becomes significant; a large proportion of that rotamer cannot be present because a torsion angle $\theta_4 = 180°$ would not allow for the well-known parallel alignment of the two hydrocarbon chains. [b] The notation of the torsion angles is according to Sundaralingam (1972). [c] Both above and below the cmc.

observed in aqueous solvents, the splitting resulting from coupling to ^{14}N does not produce resolved lines.

Discussion

A prerequisite for our conformational analysis is that the lines are sufficiently narrow so that spin–spin interactions can be observed. This is the case for phospholipids in organic solvents or in water provided that they are present as monomers or small micelles, but not for single-bilayer vesicles (Finer et al., 1972b). The fact that the lines in the spectra of DHPC and LPPC in CD_3OD have a width at half-height comparable to that of TSS together with the observation that line widths, chemical shifts, and coupling constants are invariant over a large concentration range suggests that these lipids are present as monomers in CD_3OD. In $CDCl_3$ and $CDCl_3$–CD_3OD, phosphatidylcholines have been reported to form small micelles containing 60–70 molecules (Dervichian, 1964). The line widths observed in the spectrum of DPPC in $CDCl_3$–CD_3OD (2:1 v/v) are consistent with that finding. Similar line widths are observed with DHPC and LPPC in D_2O at concentrations greater than the cmc indicating that these lipids also aggregate to small micelles in water. An approximate estimate of the micelle size (weight) was obtained from analytical gel filtration and ultracentrifugation. The apparent Stokes diameter of the DHPC micelle determined by gel filtration on Sepharose 4B is ~25 Å, and the micelle weight of LPPC determined by meniscus depletion sedimentation equilibrium is 28.5×10^3, corresponding to about 65 molecules per micelle.

The assumptions involved in the calculation of fractional populations using vicinal spin–spin coupling constants were discussed in detail previously (Hauser et al., 1978b, 1980). The fractional populations of the three staggered conformations which are assumed to be minimum free energy conformations are summarized in Tables IV and V, and the results may be compared with the LPPC conformation in the crystal structure (Figure 6). The following conclusions can be drawn. (1) The phosphorylcholine group of all compounds investigated has a distinct preferred conformation with torsion angles α_1 and α_4 in the range 150–160° (see below) and α_5 = ±gauche. (2) The preferred conformation of the phosphorylcholine group

Table V: Minimum Energy Conformation of the Polar Group of 1-Lauroylpropanediol-3-phosphorylcholine

bond	torsion angle[a]	definition[a]	conformation	fractional population: LPPC in D_2O	fractional population: LPPC in CD_3OD
$R_1CO_2CH_2-\langle CH_2CH_2OP$	θ_3	C1-C2-C3-O31	±gauche	0.64	0.67
			antiplanar	0.36	0.33
$CH_2CH_2-\langle CH_2OP$	θ_1	O11-C1-C2-C3	±gauche	0.70	0.67
			antiplanar	0.30	0.33
$CH_2CH_2-\langle OP$	α_1	C2-C1-O11-P	±gauche	0.32	0.28
			antiplanar	0.68	0.72
$PO-\langle CH_2CH_2N$	α_4	P-O12-C11-C12	±gauche	0.22	0.26
			antiplanar	0.78	0.74
$POCH_2-\langle CH_2N$	α_5	O12-C11-C12-N	±gauche	1.00	1.00
			antiplanar	0.00	0.00

[a] The notation of the torsion angles and the numbering of the atoms is according to Sundaralingam (1972).

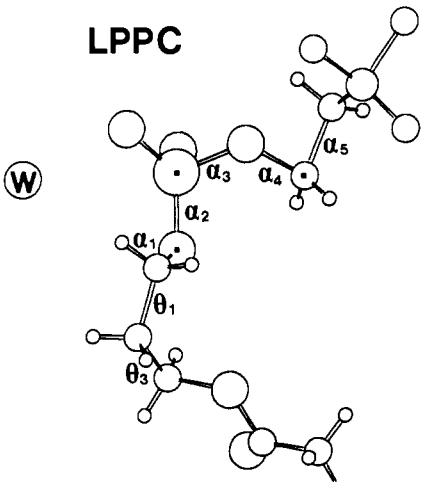

FIGURE 6: Single-crystal structure of the polar group of LPPC; a full account of this work is given elsewhere (Hauser et al., 1980). Atoms labeled (■) are located in the plane of projection. (Ⓦ) designates the position of one water molecule of hydration. The values of the torsion angles are $\alpha_1 = \pm 162°$, $\alpha_2 = \pm 86°$, $\alpha_3 = \pm 45°$, $\alpha_4 = \pm 129°$, $\alpha_5 = \pm 84°$; $\theta_1 = \pm 28°$; $\theta_3 = \pm 78°$.

is independent of the number of fatty acyl chains [cf. Hauser et al. (1978b)]. (3) Solvation cannot be a determining factor because replacement of water of hydration by CD_3OD or $CDCl_3$–CD_3OD had no effect on the conformation of phosphorylcholine. (4) The conformation of the phosphorylcholine group in DHPC is the same in the monomeric state and in the micellar aggregate. (5) Except for some differences in torsion angle α_4 which appears to be closer to antiplanar when the lipids are present in solution, the preferred conformation of the phosphorylcholine group in solution is in good agreement with the crystal structure (Figure 6). The fact that the conformation of phosphorylcholine is independent of the solvent and of the state of aggregation indicates that the conformation is primarily determined by intramolecular forces.

The quintet and the complex multiplet observed for the CH_2N and $POCH_2$ (choline) groups, respectively (Figures 1b,c–5b,c), clearly indicate that there is a conformational preference about the O–C–C–N bond. For all compounds studied (cf. Tables IV and V), the torsion angle α_5 defining that bond is almost exclusively gauche. This is consistent with NMR studies on dipalmitoylphosphatidylcholine and phosphatidylethanolamine in organic solvents (Birdsall et al., 1972; Dufourcq & Lussan, 1972) and with NMR work on lysophosphatidylcholine in H_2O (Hauser et al., 1978b) and on phospholipid constituents and choline derivatives (Dufourcq & Lussan, 1972; Partington et al., 1972). A strongly preferred gauche conformation for the O–C–C–N bond is also observed in the crystal structures of LPPC (Figure 6), of 1,2-dilauroylphosphatidylethanolamine (Hitchcock et al., 1974), and of various constituents of phospholipids (Abrahamsson & Pascher, 1966; Sundaralingam, 1972).

The values of the vicinal ^{14}N–^1H coupling constants observed for phosphatidylcholine in organic solvents (Table III) are in the range of 2.5–2.8 Hz. By use of the angular dependence of the ^{14}N–^1H spin coupling, it can be shown that values of $^3J_{NH} \geq 2.5$ are typical for a predominantly gauche conformation (Terui et al., 1968; Partington et al., 1972), entirely consistent with the conclusions derived from ^1H–^1H spin coupling. The smaller value of the ^{14}N–^1H spin coupling constant observed for DHPC and LPPC in D_2O (cf. Table III) is explained by postulating that the ^{14}N nucleus relaxes sufficiently fast under these conditions so that it is partially decoupled.

In the ±gauche conformation of the fragment O–C–C–N, the positively charged ammonium group and the anionic phosphate oxygen are on the same side relative to the rest of the molecule so that the ^+N–O^- distance is close to the van der Waals distance (Sundaralingam, 1968). This suggests that intramolecular, electrostatic interaction is the major contribution to the stabilization of the gauche conformation. An approximate estimate of the free energy involved can be obtained from the gauche to antiplanar ratio of torsion angle α_5. In the compounds summarized in Tables IV and V, the antiplanar rotamer is present to less than 2%, and the free energy needed to produce such a distribution is at least −2.7 kcal/mol. A gauche ⇌ antiplanar conformational interconversion of the O–C–C–N fragment of phosphatidylcholine and its lyso derivative was only observed in the presence of trivalent lanthanides (Hauser et al., 1976, 1978b).

The ^{31}P nucleus is coupled to its adjacent CH_2 protons of the glycerol and the choline group: the ^{31}P–^1H spin coupling constants $^3J_{PH}$ are close to 6 Hz (range 5.7–7.5 Hz), corresponding to predominantly antiplanar conformations about the C–C–O–P bond (torsion angle α_1) and the P–O–C–C bond (torsion angle α_4). This conclusion is supported by ^{13}C NMR measurements from which the vicinal ^{31}P–^{13}C spin coupling constants ($^3J_{PC}$) are derived. By use of the approximate angular dependence for $^3J_{PC}$ [cf. Lapper & Smith (1973)], the motionally averaged values thus obtained for α_1 and α_4 are between 150 and 160°, in good agreement with the results of the ^{31}P–^1H spin coupling.

No information concerning the conformation about the P–O ester bonds (torsion angles α_2 and α_3) can be deduced from spin–spin coupling constants as discussed above. Our knowledge of these torsion angles is mainly based on crystal structures. The most preferred conformations about the P–O bonds of the phosphodiester group are in the ±gauche range

(Sundaralingam, 1972). By analogy with the crystal structures, it is likely that the preferred conformation in solution is also in the ±gauche range. With this assumption, the orientation of the phosphorylcholine group is approximately perpendicular to the glycerol group or parallel with respect to the lipid–water interface, i.e., to the plane of the bilayer. This is consistent with the conformation of the phosphorylcholine group in unsonicated dipalmitoylphosphatidylcholine bilayers at different states of hydration and both below and above the transition temperature as deduced from ^{31}P NMR studies (Seelig & Gally, 1976; Seelig et al., 1977; Kohler & Klein, 1976; Herzfeld et al., 1978; Griffin et al., 1978), from deuteron NMR (Seelig et al., 1977), and from neutron diffraction using selectively deuterated dipalmitoylphosphatidylcholines (Büldt et al., 1978). From a comparison of our data with the conformation of phosphatidylcholine in hydrated bilayers, it is concluded that within the error of the measurement the motionally averaged conformation of the phosphatidylcholine polar group is the same in the monomeric, micellar, and bilayer states.

There is a significant difference in the conformation between the glycerol group of diacylphosphatidylcholines and the propanediol group of LPPC (cf. Tables IV and V). No conformational preference about the two C–C bonds of the 1,3-propanediol moiety (torsion angles θ_1 and θ_3) is observed for LPPC, i.e., the three possible rotamers are nearly equally populated (Table V). This is contrasted by the behavior of lysophosphatidylcholines as discussed before (Hauser et al., 1978b) and diacylphosphatidylcholines (cf. Tables IV and V). For the latter class of compounds preferred conformations about the two C–C bonds of the glycerol group are observed both in D_2O and in organic solvents. Those conformations (torsion angles θ_3 and θ_4) which allow intra- and intermolecular chain stacking are favored. For instance, a significant proportion of the possible staggered rotamers about the glycerol C–C bonds exhibits a conformation consistent with that found in the crystal structure of 1,2-dilauroylphosphatidylethanolamine (Hitchcock et al., 1974; Hauser et al., 1978a) with torsion angles θ_1 (θ_2) = –gauche (+gauche) and θ_3 (θ_4) = antiplanar (+gauche). Torsion angles θ_1 and θ_2 determine the orientation of the phosphorylcholine group relative to the two hydrocarbon chains; the fractional populations (cf. θ_1 and θ_2 in Table IV) indicate that, similar to LPPC (cf. Table V), the diacylphosphatidylcholine molecule is quite flexible about the O11–C1–C2–C3 bond (torsion angle θ_1).

We want to emphasize that the conformation of the glycerylphosphorylcholine group of DHPC in H_2O at concentrations less than the cmc is very similar to that observed in the micelle (greater than the cmc) as indicated by almost identical sets of coupling constants (cf. Table III). This supports the notion that the polar group conformation of phosphatidylcholine is primarily determined by the intramolecular energetics of the compound. The principle governing the conformation about the C1–C2–C3–O31 bond (torsion angles θ_3 and θ_4) is the parallel alignment of the hydrocarbon chains optimizing hydrophobic interactions. The similarity in conformation of the glycerol group (torsion angles θ_3 and θ_4) of DHPC in D_2O below and above the cmc indicate that chain stacking is also energetically favored in the monomeric state. There are, however, small differences in the vicinal coupling constants J_{AC} and J_{BC} of DHPC below and above the cmc, leading to slightly different fractional populations of torsion angles θ_3 and θ_4. Packing of DHPC in micelles apparently increases the fractional population with torsion angles θ_3 (θ_4) = antiplanar (+gauche) which is the conformation found in the crystal structure of 1,2-dilauroylphosphatidylethanolamine.

The preferred conformation about torsion angles θ_3 (θ_4) manifests itself in the nonequivalence of the two α-methylene protons in phosphatidylcholine. (1) In all diacyl compounds studied the two α-methylene protons have different chemical shifts. (2) The two α-methylene protons of chain 1 (*sn*-1 position) are apparently chemically and magnetically equivalent, their chemical shift (Table II) reflecting a more hydrophobic environment as compared to the α-methylene protons of chain 2 (*sn*-2 position). (3) The two α-methylene protons on chain 2 are chemically nonequivalent, and their chemical shifts and accessibility to lanthanides (Hauser et al., 1978a) suggest that they are closer to the lipid–water interface. These findings are entirely consistent with the conclusions of Dennis and co-workers (Roberts & Dennis, 1977; Roberts et al., 1978) and with Raman spectroscopic data (Gaber et al., 1978). The spectral differences observed between the two α-methylene groups require a phospholipid structure in which the attachment of chain 1 to the glycerol is different from that of chain 2. This is, for instance, the case in the crystal structure of 1,2-dilauroylphosphatidylethanolamine (Hitchcock et al., 1974) in which the first two C atoms of chain 2 are oriented parallel to the bilayer surface. These C atoms are closer to the aqueous phase than the first few C atoms of chain 1 which are oriented perpendicular to the bilayer surface. The conformational analysis presented here suggests that a similar mode of hydrocarbon chain packing is predominant in phosphatidylcholines. Work is currently in progress to identify and characterize other modes of chain stacking consistent with the spectral nonequivalence of the two hydrocarbon chains.

Acknowledgments

1-Lauroylpropanediol-3-phosphorylcholine (LPPC) was synthesized by R. Berchtold, Biochemisches Labor, 3007 Bern, Switzerland.

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Mechanism of Action of Milk Lipoprotein Lipase at Substrate Interfaces: Effects of Apolipoproteins†

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ABSTRACT: The mechanism of action of bovine milk lipoprotein lipase was studied by using a monomolecular film of 1,2-didecanoylglycerol. The apparent rate of hydrolysis of diglyceride increased with increasing surface pressures above 12 mN/m; the enzyme was inactive at pressures less than 12 mN/m. We have measured the effects of four plasma apolipoproteins (apoC-II, apoC-III, apoA-I, and apoE), bovine serum albumin, porcine pancreatic colipase, heparin, and NaCl on the kinetics of lipid hydrolysis. At a surface pressure of 15 mN/m, all of the proteins, with the exception of colipase, gave increased enzyme activity compared to lipase alone; apoC-II gave maximal activation. At 25 mN/m, apoC-II at concentrations of less than 0.25 μg/mL showed a specific activation, whereas the other proteins had no effect. Heparin activated at both high and low surface pressures; NaCl had little or no effect in this system. At a higher concentration of apoC-II (0.50 μg/mL), the apoprotein inhibited the enzyme. The addition of apoC-III, apoA-I, or apoE (final concentration 0.25 μg/mL), but not albumin or colipase, to apoC-II (0.25 μg/mL) caused an increase in surface pressure of 5-6 mN/m and an apparent rate which was less than half that found for lipase alone, suggesting that all of the apoproteins inhibit the apoC-II specific activation.

Lipoprotein lipase, glycerol ester hydrolase (EC 3.1.1.3), hydrolyzes plasma triglycerides which are transported in the circulation by chylomicrons and very low density lipoproteins (VLDL)[1]. The enzyme is located at the vascular endothelium and is rapidly released into plasma by intravenous injection of heparin (Korn, 1955). Lipoprotein lipase is found at high levels in adipose tissue, muscle, and lactating mammary gland. A number of investigators [for a review, see Fielding & Havel (1977)] have shown that apolipoprotein C-II (apoC-II) from human VLDL is required for maximal activity of the enzyme. The effects of the other apoC proteins (apoC-I and apoC-III) and the high density apolipoproteins apoA-I, apoA-II, and apoE on the activity of lipoprotein lipase are controversial (Östlund & Iverius, 1975; Ekman & Nilsson-Ehle, 1975; Brown & Baginsky, 1972; Baginsky & Brown, 1976; LaRosa et al., 1970; Havel et al., 1973; Quarfordt et al., 1977; Ganesan & Bass, 1976; Ganesan et al., 1976). Our understanding of the structure and the mechanism of action of lipoprotein lipase at the membrane surface and its activation by apoC-II is unknown. A major reason for the lack of information concerning the detailed mechanism has been the difficulty in

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[1] Abbreviations used: VLDL, very low density lipoproteins; apoC-I, apoC-II, and apoC-III, apoproteins of VLDL; HDL, high density lipoproteins; apoA-I and apoE, apoproteins of HDL; BSA, bovine serum albumin; FA, fatty acid; LpL, lipoprotein lipase.

0006-2960/80/0419-0373$01.00/0 © 1980 American Chemical Society