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THE POLAR GROUP CONFORMATION OF 1,2-DIALKYL PHOSPHATIDYLCHOLINES

AN NMR STUDY

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The polar group conformation of 1,2-di-*O*-alkylglycerophosphocholine (dialkylphosphatidylcholine (ether phosphatidylcholine)) is investigated and compared to that of the corresponding diacylphosphatidylcholine (ester phosphatidylcholine). The motionally averaged conformation of the glycerophosphocholine group is similar in the two classes of compounds. The replacement of the ester linkages by ether bonds has therefore no significant effect on the average conformation and segmental motion of the polar group. This conclusion is based on ^{1}H spin-spin coupling constant analysis which gives information about the conformation of the glycerol (torsion angles θ_1 to θ_4, Fig. 5) and the remainder of the polar group (torsion angles $\alpha_1, \alpha_4, \alpha_5$) except for the conformation of the phosphodiester group (torsion angles α_2 and α_3). Comparison of ^{31}P powder spectra of dialkyl- and diacylphosphatidylcholine shows that the conformation of the latter group is also very similar in the two classes of compounds. The ^{1}H-NMR measurements were carried out with short chain compounds (dihexylphosphatidylcholine) which are present as monomers or small micelles in H_2O and long chain compounds (dioleylphosphatidylcholine) forming small micelles in $CHCl_3/CH_3OH$. The ^{31}P-NMR work was carried out with dipalmitylphosphatidylcholine which forms multilamellar bilayer structures in H_2O. From NMR measurements using these different structures it is clear that for both ether and ester phosphatidylcholines the average conformation and segmental motion is independent of the state of aggregation. Furthermore, displacement of water of hydration by $CHCl_3/CH_3OH$ has little effect on conformation and segmental motion. These results indicate that the motionally averaged conformation of the polar group is mainly determined by intramolecular energetics. ^{1}H-NMR provides evidence that there is almost free rotation of the phosphocholine group about the C2-C3 glycerol bond. As a result there is no preferred conformation of the phosphocholine group with respect to the diacylglycerol part of the molecule. This rotation is probably responsible for the averaging of the chemical shielding tensor leading to axially symmetric ^{31}P powder spectra as observed for aqueous phosphatidylcholine dispersions both below and above the transition temperature.

Introduction

Monoalkyl- and dialkylglycerophospholipids are naturally occuring lipids the significance of which is still unknown. Previous studies have shown that the packing of dialkyl phospholipids is similar to that of the analogous diacyl compounds [1–3].

Here we present a study of the conformation and motion of the polar group of synthetic dialkylphosphatidylcholines based upon ^{1}H high-resolution and ^{31}P broad-line NMR. Phospholipids differing in hydrocarbon chain length are used which are present in H_2O as monomers, small micelles or large multilamellar bilayer structures. By using these structures

Abbreviations: PC, phosphatidylcholine; DHPC ether, 1,2-dihexyl-*sn*-glycero-3-phosphocholine; DHPC ester, 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine; DPPC ether, 1,2-dipalmityl-*sn*-glycero-3-phosphocholine; DPPC ester, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; TSS, sodium 3-(trimethylsilyl)propanesulfonate.

the effect of aggregation upon the polar group conformation is tested. The conformation of dialkylphosphatidylcholine is compared to that of the diacyl compound.

Experimental

Materials

DHPC ether, DPPC ether and dioleyl PC (ether) were synthesized by Mr. R. Berchtold [4]. DHPC ester, DPPC ester and dioleoyl PC (ester) were synthesized as described before [1]. The lipids were pure by TLC standards.

Methods

The critical micellar concentration (CMC) of DHPC ether in H_2O was determined as described previously [5] and a value of 6.6 ± 0.6 mM was obtained. This value is less than half of the CMC of DHPC ester which was determined as 15.2 ± 1 mM [5]. ^1H high-resolution NMR spectra were recorded on a Bruker HXS-360 Fourier transform spectrometer. The conformational analysis of the ^1H-NMR spectra is based on spin-spin coupling constants which were derived from computer simulations of the ^1H-NMR spectra with an accuracy of ± 0.2 Hz. The details of the methods are given in Refs. 5, 8 and 9. ^{31}P powder-type spectra were run on a CXP-300 Fourier transform spectrometer at 121.46 MHz using unsonicated randomly oriented aqueous phospholipid dispersions. Spectra were recorded under conditions of ^1H-decoupling using a decoupling power of ~150 W. The ^{31}P powder spectra were simulated as described in Ref. 7 using Gaussian line shapes. Phospholipid dispersions were made as described in Ref. 6 or, alternatively, the solid material was dispersed in H_2O by handshaking.

Results

Proton NMR spectra of DHPC ether in H_2O below and above the CMC are shown in Figs. 1 and 2, respectively. The ^1H-NMR spectrum of DHPC ester (published previously [5]) is shown for comparison in Fig. 3. The spectral assignment was made on the basis of homo- and heteronuclear double-resonance experiments, spectral perturbations induced by shift and broadening reagents of the lanthanide series

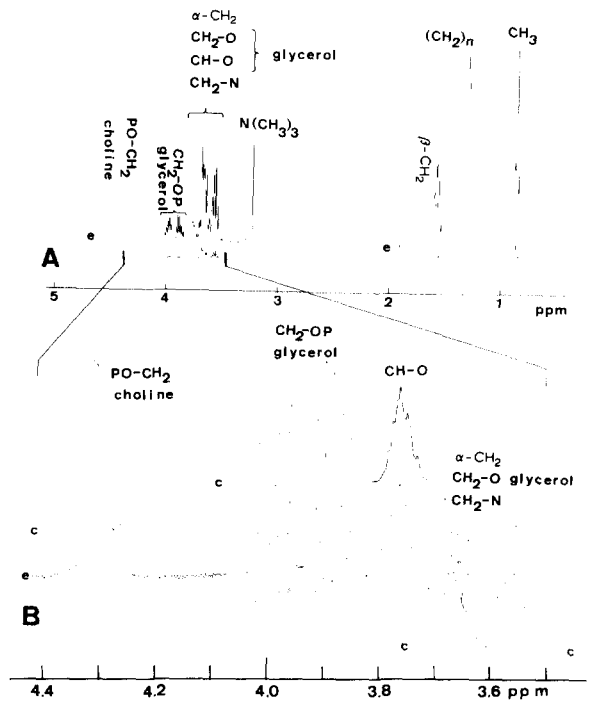


Fig. 1. 360 MHz ^1H-NMR spectra of DHPC ether in ^2H_2O below the CMC. (A) Total spectrum of DHPC ether (1 mg/ml = 2.3 mM) in ^2H_2O (nominal pH ~ 6) at 25°C. The portion of the lipid polar group indicated is horizontally expanded in Fig. 1B. The composite signal between 3.5 and 3.7 ppm from TSS consists of four α-CH_2 protons, the CH_2-N and the CH_2-O (glycerol C1) protons. Computer simulated spectra (c) are shown either above or below the experimental spectra. Letters e and c designate spectra vertically expanded and computer simulated, respectively. The computer simulation of the composite signal between 3.5–3.7 ppm in B is presented in two parts (see spectrum c below experimental spectrum): (I) the α-CH_2 protons together with the CH_2-O (glycerol C1) octet (solid line) and (II) the CH_2-N signal (dotted). It should be mentioned that the CH-O glycerol signal is a composite signal. This becomes obvious from its computer simulation. The signals from the two neighbouring CH_2-O glycerol groups are readily fitted with small RMS errors; however, when the coupling constants resulting from this fit are used to calculate the CH-O (glycerol C2) resonance no satisfactory fit is obtained. The experimental signal is more complex than the calculated one. The computed signal shown in B was obtained by mixing two components: (I) a major one (~85%) calculated with the spin coupling constants contained in Table II and (II) a minor one (~15%) calculated with $J_{AC} = 5.4$ Hz and $J_{BC} = 4.6$ Hz. This finding indicates that at least two conformations exist about the C2-C3 glycerol bond which are long-lived on the NMR time scale. The origin of the minor component contributing to the CH-O signal is not known.

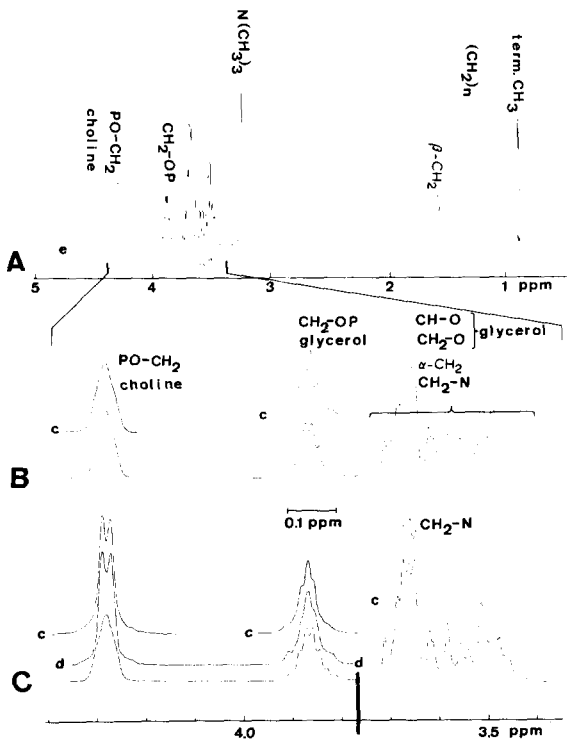


Fig. 2. 360 MHz ^{1}H-NMR spectra of DHPC ether in ^2H_2O above the CMC. (A) Total spectrum of DHPC ether (110 mg/ml = 0.248 M) in ^2H_2O (nominal pH ~ 6) at 25°C. The part of the lipid polar group indicated is horizontally expanded in (B). The same spectral region as in (B) is shown in (C) under conditions of double resonance. On the left of the separating line the PO-CH_2 (choline) and CH_2-OP (glycerol) are shown when the CH-O (glycerol C2) and the CH_2-N protons were irradiated simultaneously (d). The computer simulation c is given on top. On the right the region between 3.5 and 3.8 is given with the β-CH_2 protons of the hydrocarbon chains at 1.5–1.6 ppm being irradiated. Under these conditions both α-CH_2 signals which are originally ABX_2 multiplets collapsed to AB quartets. The position of the CH_2-N signal becomes obvious and its computer simulation c is given on top.

and by comparison with the ^{1}H-NMR spectra of DHPC ester. The ether differs from ester in the chemical shifts of the glycerol and the α-CH_2 chain protons next to the glycerol backbone. The α-CH_2 protons of the ether PC (Figs. 1 and 2) overlap the polar group signals. The two protons of the α-CH_2 group attached to glycerol C2 are non-equivalent resonating at 3.63 and 3.70 ppm from TSS; the other two α-CH_2 protons are also non-equivalent resonating at 3.55 and 3.58 ppm (Figs. 1 and 2B). In contrast, the α-CH_2 protons of DHPC ester resonate upfield of the polar group resonances: the α-CH_2 protons attached to glycerol C2 at 2.40 and 2.43 ppm from TSS and the α-CH_2 protons of the glycerol C1 at 2.36 ppm (Fig. 3). These differences in chemical shift of the α-CH_2 and glycerol protons account for the difference in the spectra of the two compounds (cf. Figs. 1 to 3). The chemical shifts of the choline signals and the remainder of the hydrocarbon chain signals of DHPC ether are very similar to those of DHPC ester. Upon aggregation (micellisation) of DHPC ether, the terminal CH_3 changed from a single triplet to two partially overlapping triplets as shown by computer simulation of the CH_3 signal (data not shown). The chemical non-equivalence of the two hydrocarbon chains is apparently amplified in the micellar aggregate. Micelle formation was also accompanied by slight

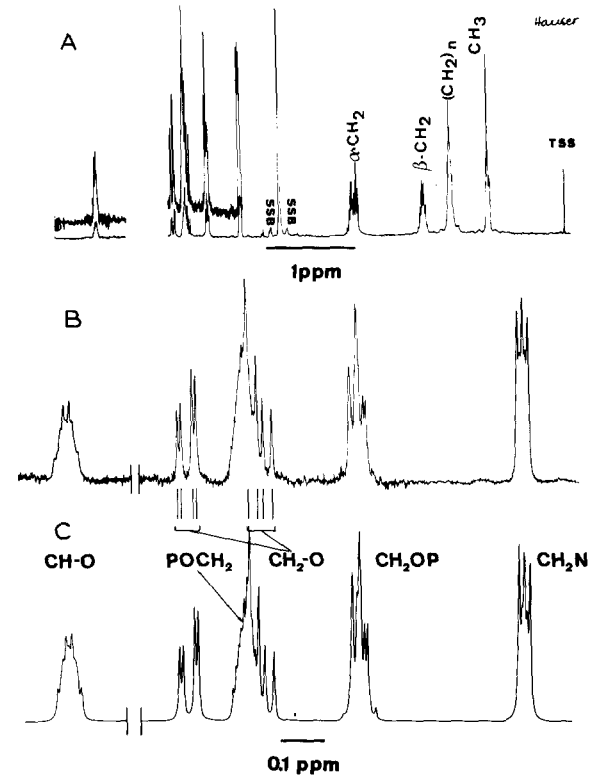
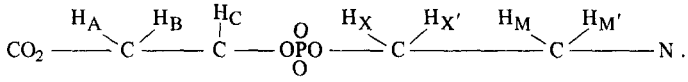


Fig. 3. 360 MHz ^{1}H-NMR spectrum of DHPC ester in ^2H_2O at a concentration > CMC. (A) Total spectrum at 10 mg/ml (=21.2 mM) in ^2H_2O (nominal pH ~ 6) at 25°C, (B) expanded spectrum of the polar group except for the $N(CH_3)_3$ signal and (C) its computer simulation. SSB, spinning side band.

TABLE I

CHEMICAL SHIFTS

Chemical shifts are expressed in ppm downfield from TSS as internal standard. The lettering used to designate the protons of the polar group is as before [5]:



Group	Signal		DHPC ether in ^2H_2O < CMC	DHPC ether in ^2H_2O > CMC	DHPC ester in ^2H_2O > CMC [a]
Glycerol	CH_2–O	H_A	3.56	3.6 –3.8	4.29
		H_B	3.66		4.47
	CH–O	H_C	3.77	3.70	5.34
	CH_2–OP	H_D	3.89	3.85	4.07
		H_E	3.99	3.88	4.08
Choline	PO–CH_2	H_X		4.28	
		$H_{X'}$	4.30	4.30	4.33
	CH_2–N	H_M	3.69	3.66	3.71
		$H_{M'}$			
	$N(CH_3)_3$		3.22	3.22	3.27

[a] Chemical shifts of DHPC ester were published before [5] and are included for comparison.

TABLE II

SPIN-SPIN COUPLING CONSTANTS OF THE POLAR GROUP SIGNALS OF DHPC ETHER AND DHPC ESTER

Signal	Spin-coupling constant (notation)	Spin-coupling constants		
		DHPC ether in ^2H_2O < CMC	DHPC ether in ^2H_2O > CMC	DHPC ester in ^2H_2O > CMC
CH_2O	$^2J_{AB}$	11.6	10.6 [a]	12.2
	$^3J_{AC}$	5.6–6.5	6.5 [a]	7.5
	$^3J_{BC}$	3.3–3.8	3.0 [a]	2.7
CH–O	$^3J_{CD}$	5.9	5.6	5.6
	$^3J_{CE}$	4.0	4.4	3.8
	$^2J_{DE}$	11.3	10.9	6.8
CH_2–OP	$^3J_{PH_D}$	5.3	4.9	7.1
	$^3J_{PH_E}$	5.1	6.1	5.9
OP–CH_2	$^2J_{XX'}$	14.0	15.2	12.0
	$^3J_{PH_X} = {}^3J_{PH_{X'}}$	6.5	6.15	6.5
	$^3J_{NH_X} = {}^3J_{NH_{X'}}$	2.7	1.5	1.5–1.7
CH_2–N	$^2J_{MM'}$	14.0	15.2	12.0
	$^3J_{MX} = {}^3J_{M'X'}$	2.3	2.4	2.5
	$^3J_{MX'} = {}^3J_{M'X}$	6.8	6.9	6.7

[a] These coupling constants could not be derived from the original spectra due to overlapping with the multiplets of the CH_2-N and CH-O signals. In the presence of $Pr(NO_3)_3$ (~60 mM) the CH_2-N and CH-O signals were shifted downfield sufficiently to reveal the octet of the CH_2-O group. Under these conditions the coupling constans were readily derived from the computer simulation of the octet.

changes in chemical shift of the glycerol signals while the signals from the choline group were unaffected (Table I).

Spin-coupling constants were derived from the computer-simulated spectra (Figs. 1 to 3B and C) as described before [5,8,9] and those of the polar group signals are summarized in Table II. Although there are differences in chemical shifts between ether and ester PC (cf. Figs. 1–3) as discussed above the spin-coupling constants of the two compounds are rather similar (Table II). For both DHPC ether (cf. Table II) and ester the hyperfine structure of the spectra and hence the spin-coupling constants did not change appreciably upon micelle formation. Furthermore, the spin-coupling constants of dioleyl PC (ether) in $C^2HCl_3/C^2H_3O^2H$ (2 : 1, v/v) were very similar to those of dioleoyl PC (ester) in the same solvent (data not shown). The spin coupling constants measured for the long-chain compounds in organic solvent were close to those in Table II. These results indicate that for both classes of compounds the conformation and segmental motion of the polar group are similar. Furthermore, these properties appear to be insensitive to solvent polarity and to micellar aggregation. The vicinal spin coupling constants summarized in Table II were used to compute fractional populations for the three staggered conformations of minimum free energy as discussed before [5,8,9]. The results for the C-C and C-O bonds of DHPC (ether) are summarized in Table III.

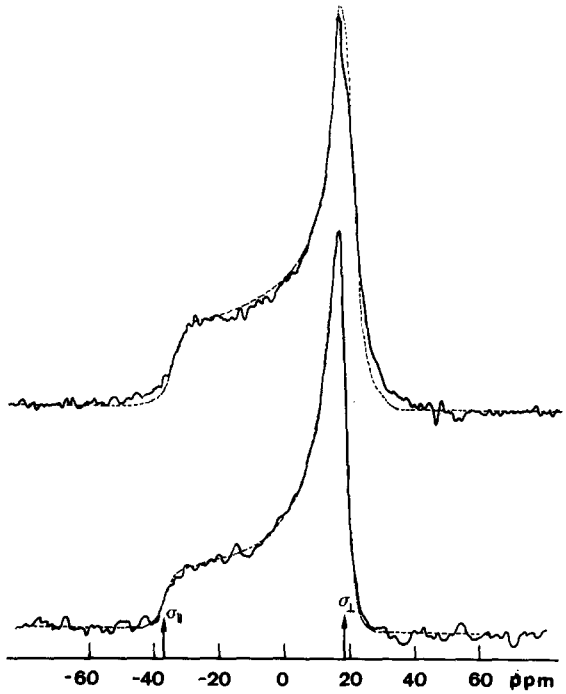


Fig. 4. Proton decoupled ^{31}P-NMR spectra of unsonicated aqueous dispersions of DPPC ester (top) and DPPC ether (bottom). The lipids were dispersed in H_2O at a concentration of approx. 80 mg/ml and ^{31}P spectra were recorded at 31 ± 1°C and at 121.46 MHz using a Bruker CXP 300 Fourier transform spectrometer. Approximately 2500 free induction decays were averaged to obtain the spectra shown. Protons were decoupled using a proton-decoupling power of ~150 W. Chemical shifts are referenced to external 85% orthophosphoric acid. The chemical shift anisotropy, $\Delta\delta = \delta_\parallel - \delta_\perp$ is given, to a first approximation, by the edges of the powder spectrum. The value derived from the computer simulation of the spectra is $\Delta\delta = -56 \pm 2$ ppm and the tensor components are $\delta_\parallel = -37$ ppm and $\delta_\perp = 18.5$ ppm. The linewidth of the individual Gaussian line used in the fit is Δ = 270 Hz in good agreement with values in the literature [20].

The proton-decoupled ^{31}P-NMR spectra obtained from unsonicated, aqueous dispersions of DPPC ether and ester were almost superimposible. This was true at temperatures below the transition point T_c (Fig. 4) as well as above (data not shown). Spectra were recorded at 121.46 MHz under conditions of proton decoupling using a decoupling power of ~150 W. The values for the chemical shift anisotropy derived from the computer simulated spectra were $|\Delta\delta| = 58 \pm 2$ ppm at 32°C and 49 ± 2 ppm at 50°C. The temperature dependence of $\Delta\delta$ was in good agreement with the work of Seelig and his collaborators on DPPC ester [7]. Above the T_c temperature similar results were obtained at 36.4 MHz with a decoupling power of only 10–15 W, but below the T_c the line shape was distorted under these conditions due to residual ^{1}H-^{31}P dipolar coupling.

Discussion

Conformational information has been derived from ^{1}H-NMR using mainly short chain dialkyl PC and ^{31}P-NMR using long chain dialkyl PC. Similar to DHPC ester [5], DHPC ether forms small micelles in ^2H_2O at concentrations above the CMC which give rise to well resolved high resolution NMR spectra with lines narrow enough to reveal spin-spin coupling. The ^{31}P-NMR work was carried out with unsonicated,

TABLE III

THE MINIMUM FREE ENERGY CONFORMATION OF THE POLAR GROUP OF DHPC ETHER IS COMPARED TO THAT OF DHPC ESTER

Bond	Torsion [a] angle	Staggered [b] conformation	Fractional population		
			DHPC ether in ^2H_2O < CMC	DHPC ether in ^2H_2O > CMC	DHPC ester [c] in ^2H_2O
$R_1COCH_2-CHOCR_2$	$\theta_3(\theta_4)$ [d]	+*sc*(−*sc*)	0.44	0.44	0.33
		ap(+*sc*)	0.44	0.50	0.60
		−*sc*(*ap*)	0.12	0.06	0.03
$R_2COCH-CH_2OP$	$\theta_1(\theta_2)$ [d]	−*sc*(*sc*)	0.44 (0.49)	0.43 (0.49)	0.48 (0.53)
		ap(−*sc*)	0.37 (0.15)	0.37 (0.15)	0.37 (0.13)
		sc(*ap*)	0.19 (0.36)	0.20 (0.36)	0.15 (0.34)
$CHCH_2-OP$	α_1	±*sc*	0.16	0.16	0.23
		ap	0.84	0.84	0.77
$PO-CH_2CH_2$	α_4	±*sc*	0.24	0.24	0.26
		ap	0.76	0.76	0.74
$POCH_2-CH_2N$	α_5	+*sc*	1.00	1.00	0.98
		ap	0.00	0.00	0.02

[a] The notation of the torsion angles is according to Sundaralingam [10].

[b] The nomenclature used to describe staggered rotamers is that of Klyne and Prelog [11]: *sc* = *synclinal* or *gauche* (=60 ± 30°) *ap* = *antiperiplanar* (=180 ± 30°).

[c] Both below and above the CMC. The results for DHPC ester [5] were included for comparison and are valid both below and above the CMC.

[d] For torsion angles θ_3 and θ_4 the set of values presented is obtained for $J_{AC} > J_{BC}$. The alternative solution with $J_{AC} < J_{BC}$ can be ruled out because the rotamer with $\theta_4 = ap$ would become significant. The torsion angle $\theta_4 = ap$ does not allow the typical parallel stacking of the two hydrocarbon chains and represents therefore an unfavourable conformation. For torsion angles θ_1 and θ_2 two sets of values were obtained with $J_{CD} > J_{CE}$ and with $J_{CD} < J_{CE}$ (in parenthesis).

aqueous dispersions of DPPC ether or ester in which the lipid is known to be present as multilamellar bilayers. This type of structure gives rise to powder-type NMR spectra. The main conclusion derived from the two independent NMR methods is that in terms of conformation and segmental motion of the polar group dialkyl PC is very similar to or the same as the corresponding diacyl compound. The replacement of the ester linkages in diacyl PC by ether bonds therefore does not significantly affect the conformation and motion of the glycerylphosphocholine group. This is true for short chain PCs being present as monomers or small micelles in organic solvents or ^2H_2O as well as for long chain PCs forming small micelles in organic solvent and bilayers in aqueous dispersions. Comparison of the ^{1}H-NMR spectra of DHPC ether in ^2H_2O recorded above and below the CMC indicates that, as with DHPC ester [5], the polar group conformation is by and large, independent of the state of aggregation (cf. Figs. 1 and 2, Table II and III). Furthermore, the main features of the polar group conformation are preserved when H_2O as the solvent is replaced by organic solvents such as CH_3OH and mixtures of CH_3OH and $CHCl_3$. These findings indicate that the motionally averaged conformation is mainly determined by intramolecular energetics [5].

The conformational details of the polar group of PC as derived from ^{1}H high-resolution NMR [5,8,9] and from ^{31}P broad-line NMR [7,12,13] were discussed before and need not be repeated here. In summary, the N-C-C-O bond of the choline group is characterized by an almost exclusively synclinal conformation while torsion angles α_1 and α_4 are antiperiplanar (cf. Table III). ^{1}H-NMR gives no information on the conformation of the phospho-

diester group. Conclusions concerning the conformation of that group can, however, be derived from ^{31}P powder spectra which, in this respect, complement the ^{1}H-NMR measurement. The ^{31}P-NMR spectra in Fig. 4 are typical for an axially symmetric chemical shielding tensor [14,15] and the DPPC ester spectrum has been interpreted to be consistent with an orientation of the phosphocholine group parallel to the bilayer plane [13]. By comparison (cf. Fig. 4) it is clear that the same conclusion holds for the DPPC ether.

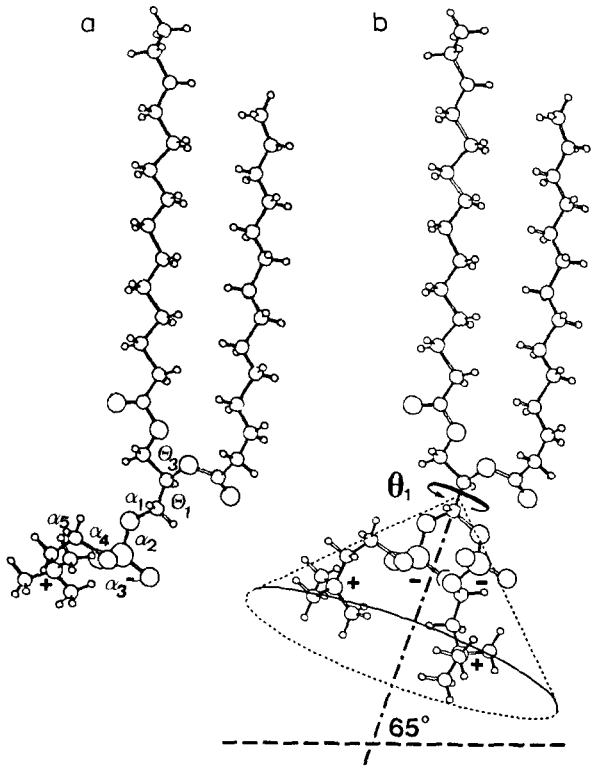


Fig. 5. (a) Conformation of 1,2-dimyristoyl PC as derived from the single crystal structure [17,19]. (b) The head group of the PC molecule shown in a is rotated about the C2-C3 glycerol bond (torsion angle θ_1) with torsion angles α_1 to α_6 being fixed. The head group sweeps out the cone shown which is tilted with respect to the bilayer normal by about 25°C; the angle between the C2-C3 axis and the plane of the bilayer is 65°. The two orientations shown within the cone correspond to torsion angles θ_1 = *+synclinal* (left) and θ_1 = *antiperiplanar* (right).

From the ^{1}H-NMR data it is clear that there are two different conformations about the C1-C2 glycerol bond (torsion angles θ_3, θ_4) which are almost equally populated (Table III). Both conformations allow the parallel stacking of the two hydrocarbon chains. One conformation (θ_3 = *ap*) is consistent with that found in the crystal structure of dilauroylphosphatidylethanolamine [16], dimyristoyl PC [17] and other lipids [18,19] and the second one (θ_3 = *sc*) is found in the single crystal structure of a diacylglycerol [21]. In contrast to the C1-C2 glycerol bond, there is nearly free rotation about the C2-C3 glycerol bond. As a result there is no preferred orientation of the phosphocholine group relative to the diacylglycerol part of the phospholipid molecule (cf. Fig. 5). In Fig. 5 the effect of rotation of the head group about the C2-C3 glycerol bond (torsion angle θ_1) is shown. It is seen that the head group with torsion angles α_1–α_5 [10] being fixed sweeps out a cone with the C2-C3 bond as the axis [19]. In this context it is interesting to note that the crystal structures of various phospholipids differ only in the conformation of the C2-C3 bond (torsion angle θ_1) [19]. In these crystal structures the conformation about the C2-C3 bond varies between nearly eclipsed and antiperiplanar [19] whereas all the other torsion angles defining the polar group lie within a rather narrow range [19].

The finding that in monomers and micelles there is free rotation about the C2-C3 bond is very likely to be true also in phospholipid bilayers. Such a rotation is consistent with the ^{31}P-NMR spectra of unsonicated phospholipid dispersions. It has been shown [13] that unsonicated, fully hydrated, multilamellar structures of DPPC ester at temperatures slightly above –10°C give ^{31}P-NMR spectra which are typical for an axially symmetric chemical shielding tensor with an anisotropy value $\Delta\delta$ = –69 ppm. With increasing temperatures the anisotropy decreases and this decrease in $\Delta\delta$ was interpreted by Griffin and his colleagues [13] in terms of additional modes of motion. Employing the C2-C3 order parameter S = 0.66 determined by ^{2}H-NMR at 48°C [22] these authors calculated a value for $\Delta\delta = -69 \times 0.66 =$ –45.5 ppm which agrees well with the experimental value of –47 ppm [7,13]. The rotation of the head group about the C2-C3 glycerol bond described in this paper will contribute to the averaging of the chemical shielding tensor.

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