Preferred Conformation and Dynamics of the Glycerol Backbone in Phospholipids. An NMR and X-ray Single-Crystal Analysis†

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Received March 15, 1988; Revised Manuscript Received July 11, 1988

ABSTRACT: The conformation of the glycerol group of a number of diacyl and monoacyl (lyso) phospholipids differing in the chemical nature of the head group was studied by ^1H high-resolution NMR and X-ray crystallography. The NMR measurements were carried out with solutions or micellar dispersions of the lipids in deuteriated organic solvents or ^2H_2O. Both solutions, in which the lipid is present as monomers, and lipid micelles give rise to good high-resolution NMR spectra exhibiting spin coupling hyperfine interactions. From ^1H spin coupling it is concluded that there are two stable conformations about the glycerol C(2)–C(3) bond of phospholipids. One of these (rotamer A) is characterized by torsion angles θ_3 = antiperiplanar, θ_4 = +synclinal, and the other (rotamer B) by θ_3 = +synclinal, θ_4 = −synclinal. In both rotamers A and B the ester oxygens on the glycerol carbon atoms C(2) and C(3) are synclinal, and hence both types of rotamers readily allow the parallel alignment of the two hydrocarbon chains. By comparison of NMR and single-crystal X-ray data it is obvious that both conformations are minimum free energy conformations. Rotamer A is the conformation prevailing in phospholipid single-crystal structures. The conformation of rotamer B is also found in phospholipid single-crystal structures though to a lesser extent, e.g., in 2,3-dilauroyl-DL-glycero-1-phospho-*N*,*N*-dimethylethanolamine and 2,3-dimyristoyl-D-glycero-phospho-DL-glycerol. NMR measurements indicate that in liquid crystals the diacylglycerol part of phospholipids fluctuates between the two stable staggered conformations of rotamers A and B. The transition between rotamers A and B is fast on the NMR time scale and must be accompanied by appropriate changes in the torsion angles β_1 to β_4 and γ_1 to γ_4 of the two fatty acyl chains. The changes in these torsion angles are such that the parallel alignment of the fatty acyl chains is warranted. From examination of the single-crystal structures of phospholipids, the minimum changes in torsion angles β_1 to β_4 and γ_1 to γ_4 accompanying the transition between rotamers A and B can be derived. It is clear from the data presented that the parallel alignment of the hydrocarbon chains or chain stacking in phospholipid aggregates such as bilayers or micelles is the fundamental principle governing the conformation of the C(2)–C(3) glycerol bond.

Phospholipids are important constituents of biological membranes, and for this reason their physicochemical properties have been studied extensively. They have a strong tendency to aggregate to bilayers, and as such they form integral structures of biological membranes. The knowledge of the structure and dynamics of phospholipids in bilayers is essential for an understanding of their functional role. The refinement of existing spectroscopic methods and the development of new ones made it possible to study the conformation and dynamics of different regions of the lipid molecule (Figure 1). The glycerol backbone can be regarded as the central part of the phospholipid molecule to which the three substituent chains are linked. The polar head group is attached to atom C(1) of the glycerol via a phosphate ester bond, and the two hydrocarbon chains are linked via ester or ether bonds to carbon atoms C(2) and C(3) of the glycerol group. For reasons discussed in detail previously (Hauser et al., 1981) we are using here Sundaralingam's (1972) atom numbering and notation for torsion angles (Figure 1). This notation necessitates that the configuration of the asymmetric carbon atom C(2) of glycerol is expressed by the D/L or by the *R*/*S* convention. In this study we address ourselves to the question of the structure and dynamics of the glycerol group in phospholipid aggregates such as micelles and bilayers. Considering the parallel stacking of the two hydrocarbon chains in lipid bilayers and lipid aggregates, a question of particular interest is whether or not this chain stacking requires a specific conformation of the glycerol group. This question is tackled by ^1H high-resolution NMR using different phospholipids and different solvents. The predominant lipid aggregate in this kind of study is the small micelle and the monomeric molecule in equilibrium with the micelle. Both give rise to high-resolution NMR spectra with lines sufficiently narrow to exhibit spin–spin interactions. Conformational information is derived from vicinal ^1H-^1H spin coupling as described before (Hauser et al., 1980a,b).

The NMR results obtained with micelles and monomeric solutions will be compared with the single-crystal structure of relevant lipids and with results in the literature obtained with NMR techniques. A comparison between precise X-ray single-crystal structures and structural data derived from liquid-crystalline, partly disordered systems will shed light on the question to what extent the minimum energy conformation of the phospholipids in the single crystals are retained in a liquid-crystalline bilayer assembly.

Materials and Methods

Various synthetic phosphatidylcholines and phosphatidylserines with saturated fatty acyl chains were synthesized as

† The work was supported by grants of the Swedish Medical Research Council (Grant 006), the Wallenberg Foundation, and the Swiss National Science Foundation (Grant 3.223-0.85).

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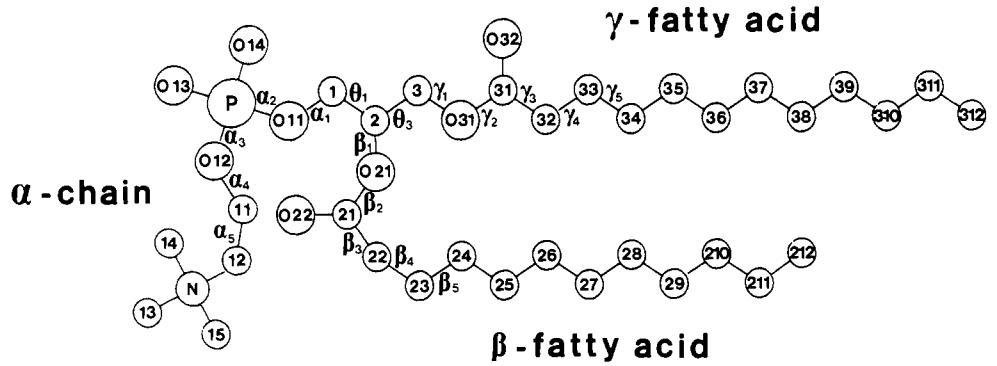

FIGURE 1: Structural notation: atom numbering and notation for torsion angles according to Sundaralingam (1972).

described previously (Paltauf et al. 1971; Hermetter et al., 1982). 2,3-Dihexyl-D-glycero-1-phosphocholine (dihexyl-PC),[1] 2,3-dilauroyl-DL-glycero-1-phospho-*N*,*N*-dimethylethanolamine ($DLPEM_2$), diacylphosphatidylethanolamines, diacylphosphatidic acids, and lysophospholipids, all with saturated fatty acyl chains, and 3-lauroylpropanediol-1-phosphocholine were synthesized by R. Berchtold (Biochemical Laboratory, Bern). The monosodium salt of 2,3-dimyristoyl-D-glycero-phospho-DL-glycerol (DMPG) was purchased from Calbiochem (CA) and egg lysophosphatidylcholine from Lipid Products (Surrey, U.K.). The phospholipids used in this study were pure as determined by TLC, C, H, N, and P microanalysis, and ^1H NMR.

Deuteriated solvents of purity 99.8% and TSS were purchased from E. Merck AG (Darmstadt, FRG). Solutions in deuteriated organic solvents and ^2H_2O and micellar lipid dispersions in ^2H_2O were prepared by weighing the solid, crystalline material, adding the appropriate volume of solvent, and dissolving or dispersing the lipid by hand shaking or vortex mixing. The apparent pH of the aqueous lipid solutions or dispersions was about 6, and if necessary, the apparent pH was adjusted to neutrality by adding small amounts of NaOD or DCl. Unless stated otherwise, the lipid concentration was 10–30 mg/mL.

Determination of the Critical Micellar Concentration (CMC). The CMC of various short-chain phospholipids and lysophospholipids in ^2H_2O was determined from changes in the fluorescence intensity of rhodamine B in the presence of increasing concentrations of phospholipid. The data were plotted as relative fluorescence intensity as a function of the logarithm of the phospholipid concentration and were best fitted by two straight lines (data not shown). The CMC was derived from the intercept of the two straight-line portions.

NMR Spectroscopy. ^1H NMR spectra of all the phospholipids and model compounds listed in Table I were recorded at 360 MHz on a Bruker HXS-360 Fourier transform spectrometer with a digital resolution of 0.18 Hz/point. All experiments were carried out at room temperature. The spectral region of the lipid polar group was computer simulated as described before (Hauser et al., 1980a), yielding ^1H spin–spin coupling constants with an accuracy of about ±0.2 Hz. It is assumed that the observed vicinal spin–spin coupling constants represent averages of the component coupling constants operating in the three staggered conformers A–C and weighted by the fractional populations of A–C (see Figure 4). On the basis of this assumption fractional populations of rotamers A–C were derived as described previously (Hauser et al., 1978, 1980). The error of ±0.2 Hz in the vicinal spin–spin coupling constants will give an error in the major fractional populations of less than ±5%. The effect of uncertainties in the component coupling constants on the fractional populations can be readily evaluated by using the law of error propagation. The major populations A and B (Figure 4) are sensitive to errors in J_{sc}^{ap} in that an error in J_{sc}^{ap} produces a similar error in the major populations A and B. These populations are, however, insensitive to errors in J_{sc}^{sc} and J_{ap}^{sc}. The minor population C is sensitive to both J_{ap}^{ap} and $J_{sc}^{sc'}$.

The determination of the single crystal of DMPC, $DLPEM_2$, DLPA, and DMPG and the details of these single-crystal structures have been described elsewhere (for references see Table III).

RESULTS

Representative ^1H NMR spectra of phospholipids dissolved or dispersed in ^2H_2O are shown in Figures 2 and 3. Figure 2A shows the ^1H NMR spectrum of the NH_4^+ salt of 2,3-dihexanoyl-D-glycero-1-phospho-L-serine (DHPS) dissolved in ^2H_2O at about 2 mg/mL (4.2 mM). This is significantly below the CMC, which was determined to be 19 ± 5 mM. The polar group region as indicated in Figure 2A is shown expanded in Figure 2C, and spectrum B is the computer simulation of the $C(3)H_2$–O glycerol and the $C(1)H_2$–OP serine ^1H signals. For comparison, the ^1H NMR spectrum of the glycerophosphocholine group of 2,3-dihexanoyl-D-glycero-1-phosphocholine (DHPC) dispersed in ^2H_2O above the CMC is presented in Figure 2D, and the computer-simulated spectrum of the polar group of DHPC is shown in Figure 2E.

Figure 3A shows the ^1H NMR spectrum of 3-lauroyl-D-glycerol 1-phosphate in ^2H_2O. An expansion of the glycerol region is shown in Figure 3B while the spectrum underneath (C) is the same region of 3-myristoyl-D-glycero-1-phosphocholine (at the same expansion). The two spectra are practically superimposable, indicating that the two lysophospholipids have identical conformation in their glycerol backbone. For comparison, the glycerol region of the ^1H NMR spectrum of egg lysophosphatidylcholine at the same expansion is shown in Figure 3D, and a computer simulation of this spectrum is given in Figure 3E. The concentration of all lysophospholipids was well above the CMC.

The assignment of the ^1H NMR spectra shown in Figures 2 and 3 was carried out as described previously (Hauser et

[1] Abbreviations: DHPC, 2,3-dihexanoyl-D-glycero-1-phosphocholine; DHPE, 2,3-dihexanoyl-D-glycero-1-phosphoethanolamine; DHPS, 2,3-dihexanoyl-D-glycero-1-phospho-L-serine; dihexyl-PC, 2,3-dihexyl-D-glycero-1-phosphocholine; DMPC, 2,3-dimyristoyl-D-glycero-1-phosphocholine; DLPA, monosodium 2,3-dilauroyl-D-glycerol 1-phosphate; $DLPEM_2$, 2,3-dilauroyl-DL-glycero-1-phospho-*N*,*N*-dimethylethanolamine; DMPG, 2,3-dimyristoyl-D-glycero-1-phospho-DL-glycerol; GPC, D-glycero-1-phosphocholine; GPE, D-glycero-1-phosphoethanolamine; TSS, sodium 3-(trimethylsilyl)propanesulfonate; sc, synclinal (gauche); ap, antiperiplanar; CMC, critical micellar concentration; TLC, thin-layer chromatography.

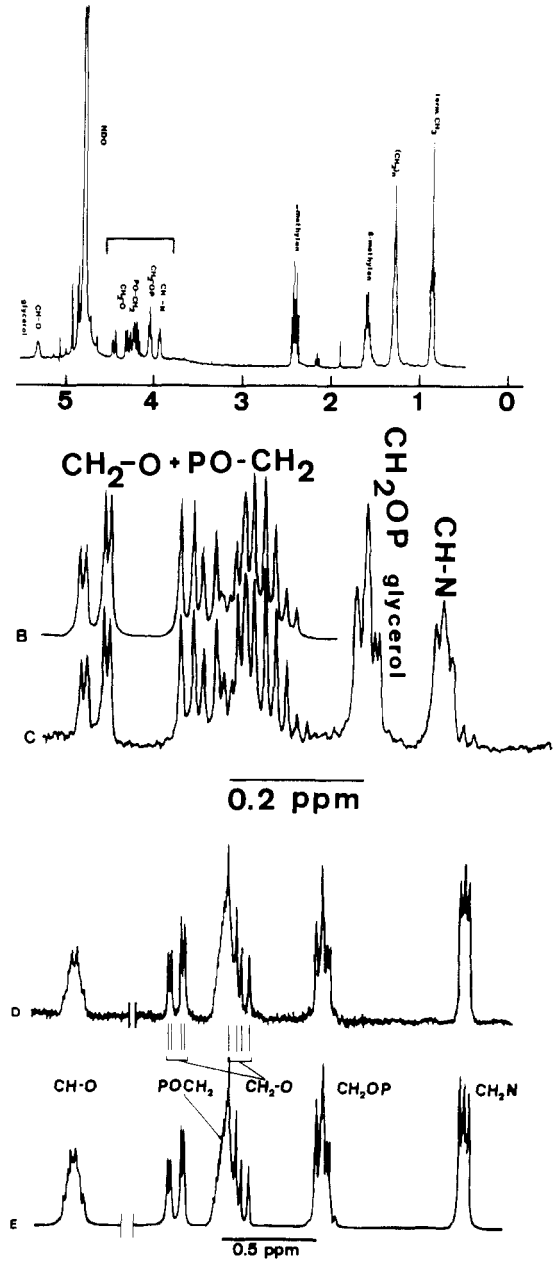

FIGURE 2: 360-MHz ^1H NMR spectrum of the NH_4^+ salt of 2,3-dihexanoyl-D-glycero-1-phospho-L-serine (DHPS) (~2 mg/mL = 4.2 mM) in ^2H_2O at a nominal pH of about 6 at 25 °C. The overall spectrum with the assignment of the signals relative to TSS is shown in (A). The polar group region within the bracket is shown expanded in (C), and the spectrum in (B) is the computer simulation of the left-hand portion of the expanded region consisting of the CH_2-O glycerol and the PO-CH_2 serine protons. (D) Expanded version of the ^1H NMR spectrum of the glycerophosphocholine group of 2,3-dihexanoyl-D-glycero-1-phosphocholine (DHPC) recorded at 25 °C. DHPC was dissolved in ^2H_2O at a nominal pH of 6 at about 10 mg/mL = 21.2 mM. This is above the CMC which was found to be 15.2 ± 1 mM. (E) Computer simulation of the spectrum shown in (D).

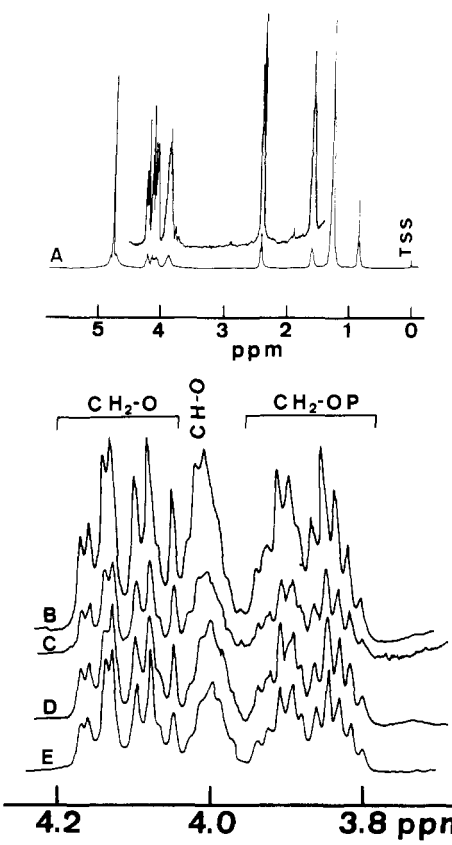

FIGURE 3: (A) 360-MHz ^1H NMR spectrum of 3-lauroyl-D-glycerol 1-phosphate (disodium salt) in ^2H_2O (~10 mg/mL = 0.025 M) at a nominal pH of 7. A vertically expanded version is shown on top of the overall spectrum. (B) Expansion of the glycerol group between about 3.8 and 4.2 ppm of the spectrum in (A). For comparison, the expanded spectra of the glycerol groups of 3-myristoyl-D-glycero-1-phosphocholine in ^2H_2O (nominal pH 6, at 10 mg/mL = 0.025 M) and of egg lysophosphatidylcholine in ^2H_2O (nominal pH 6, at ~20 mg/mL = 0.04 M) are shown in (C) and (D), respectively. The computer simulation of the glycerol group is given in (E). Spectra were recorded at 25 °C under conditions where the lysophospholipids form mainly micelles.

al., 1980a,b, 1983). The chemical shifts and vicinal spin coupling constants for the C(3)H_2–O and C(2)H–O protons of the glycerol group as obtained from the simulated spectra are summarized in Table I. With all diacyl phospholipids studied, the C(3)H_2–O glycerol protons (Figure 1) gave an eight-line spectrum characteristic of an ABX system (see for instance the eight lines marked in the spectrum in Figure 2D). Irradiation of the C(2)H–O glycerol proton reduced the octet to the four-line spectrum characteristic of the AB part. The two C(3)H_2–O protons of the glycerol are therefore both chemically and magnetically inequivalent [cf. Hauser et al. (1980a)]. This is also true for the C(3)H_2–O protons in lysophospholipids. In lysophosphatidate and lysophosphatidylcholine the C(3)H_2–O protons gave rise to a multiplet corresponding to the AB part of an ABC system (Figure 3). Furthermore, the protons of glycerol C(3) show no coupling to ^{31}P.

The protons of the C(1)H_2–OP glycerol group were usually also nonequivalent though to a lesser extent. In some cases, however, the number of lines was reduced, and deceptively simple spectra were observed. The C(1)H_2–OP glycerol protons were readily identified on the basis of ^{31}P–^1H spin coupling. This group was therefore analyzed as an ABMX or ABCX system depending on whether it belonged to a diacyl or lysophospholipid, respectively.

Since the chain stacking of the phospholipid is primarily determined by the torsion angles θ_3 and θ_4 (Figures 1 and 4), which define the conformation of the C(2)H–C(3)H_2 glycerol bond, the ^1H spin coupling constants of this part were included in Table I while those of the C(1)H_2–OP glycerol group were omitted for the sake of clarity.

Discussion

NMR Data. As discussed previously [e.g., Hauser et al. (1980a)], the lines from small phospholipid micelles are narrow enough to exhibit spin–spin coupling. In contrast, the reso-

Table I: Chemical Shifts and Coupling Constants of the C(2)H–C(3)H_2 Glycerol Protons of Different Phospholipids[a]

compound	chemical shift (ppm)			spin coupling const (Hz)			exptl conditions
	H_A	H_B	$H_{X(C)}$	$^2J_{AB}$	$^3J_{AX(AC)}$	$^3J_{BX(BC)}$	
2,3-dihexanoyl-D-glycero-1-phosphocholine	4.28	4.43	5.32	12.0	6.8	3.0	in ^2H_2O, pH 6.0, <CMC
	4.29	4.47	5.34	12.2	7.6	2.4	in ^2H_2O, pH 6.0, >CMC
	4.17	4.42	5.23	12.0	6.7	3.3	in $C^2H_3O^2H$
2,3-dihexanoyl-D-glycero-1-phosphoethanolamine	4.18	4.42	5.23	12.1	6.8	3.4	in $C^2H_3O^2H$
	4.25	4.41	5.25	12.3	7.2	3.1	in ^2H_2O, pH 6.0, <CMC, 95 °C
	4.21	4.42	5.25	12.2	7.7	2.3	in ^2H_2O, pH 6.0, >CMC, 95 °C
2,3-dihexanoyl-D-glycero-1-phospho-L-serine ammonium salt	4.28	4.43	5.31	12.3	7.1	3.0	in ^2H_2O, pH 7.5, <CMC
	4.24	4.42	5.30	12.3	7.6	2.4	in ^2H_2O, pH 7.5, >CMC
	4.20	4.44	5.23	12.0	6.6	3.2	in $C^2H_3O^2H$
2,3-di-*O*-hexyl-D-glycero-1-phosphocholine	3.56	3.66	3.77	11.6	5.6–6.5	3.3–3.8	in ^2H_2O, pH 6.0, <CMC
	3.60	3.80	3.70	10.6	6.5	3.0	in ^2H_2O, pH 6.0, >CMC
	3.48	3.57	3.64	12.2	7.5	2.7	in $C^2H_3O^2H$
2,3-dipalmitoyl-DL-glycero-1-phosphocholine	4.16	4.42	5.24	12.0	6.9	3.1	in $C^2HCl_3/C^2H_3O^2H$, 2:1 (v/v)
2,3-dipalmitoyl-DL-glycero-1-phosphoethanolamine	4.18	4.40	5.23	11.6	6.9	3.4	in $C^2HCl_3/C^2H_3O^2H$, 2:1 (v/v)
2,3-dipalmitoyl-D-glycero-1-phospho-L-serine	4.19	4.43	5.24	12.1	7.2	3.0	in $C^2HCl_3/C^2H_3O^2H$, 4:3 (v/v)
2,3-dipalmitoyl-D-glycerol 1-phosphate disodium salt	4.21	4.40	5.25	12.0	7.1	3.5	in $C^2HCl_3/C^2H_3O^2H$, 2:1 (v/v)
egg lysophosphatidylcholine	4.08	4.15	4.00	11.4	6.5	3.4	in ^2H_2O, pH 6.0, >CMC
3-myristoyl-D-glycero-1-phosphocholine	4.10	4.17	4.02	11.2	6.7	4.1	in ^2H_2O, pH 6.0, >CMC
3-lauroyl-D-glycerol 1-phosphate disodium salt	4.11	4.18	4.04	11.4	6.7	4.2	in ^2H_2O, pH 6.8, >CMC
3-lauroylpropanediol-1-phosphocholine	4.17	4.17	1.95		6.73	6.61	in ^2H_2O, pH 6.0, >CMC
	4.18	4.18	1.94		6.47	6.47	in $C^2H_3O^2H$

[a] Chemical shifts are expressed in ppm downfield from either tetramethylsilane or TSS used as an internal reference in organic solvent and ^2H_2O, respectively. Chemical shifts were measured with an accuracy of ±0.01 ppm, and spin coupling constants were derived from simulated spectra with an accuracy of about 0.2 Hz. The lettering of the glycerol protons and hence the subscripts of the coupling constants J are as described previously (Hauser et al., 1978). ^1H NMR spectra were run at 25 °C unless otherwise stated.

nances arising from phospholipid molecules present in small single-bilayer vesicles of diameter 25 nm, or even more so from large bilayer structures, are broadened to the extent that spin–spin interactions are not resolved (Penkett et al., 1968; Finer et al., 1972). Hence, the conformational analysis, which makes use of the relationship between the magnitude of vicinal proton spin coupling constants $^3J_{HH}$ and the torsion angles, is restricted to small micelles. The short-chain phospholipids and lysophospholipids used in this study form micelles when dispersed in H_2O at concentrations >CMC and are present as monomers below that concentration. According to reports in the literature it is reasonable to assume that the long-chain diacyl phospholipids also form micelles in mixtures of C^2HCl_3 and C^2H_3OH [see for instance Dervichian (1964)].

The fundamental assumption involved in the conformational analysis based on the NMR data is that the three staggered conformations about a –C–C– or a –C–O– bond represent minimum free energy conformations. The vicinal ^1H spin coupling constants $^3J_{HH}$ that operate in these three possible conformations are referred to as component coupling constants. Their values were taken from the literature (Gatti et al., 1967; Abraham & Gatti, 1969; Partington et al., 1972; Birdsall et al., 1972). Since only one set of vicinal spin coupling constants is observed for each C–C or C–O bond, there must be motional averaging between the three possible staggered conformations that is fast on the NMR time scale. The experimental vicinal spin coupling constants $^3J_{HH}$ for the C(2)–C(3) glycerol bond derived from computer simulations are summarized in Table I. They represent motional averages of the component coupling constants weighted by the fractional populations of the three staggered conformations (see Figure 4). Knowing the values of the component coupling constants, the observed vicinal spin coupling constants $^3J_{HH}$ allow us to calculate the fractional populations of rotamers for the C(2)–C(3) glycerol bond [for details see Hauser et al. (1978)]. These staggered conformations about the C(2)–C(3) glycerol bond (rotamers A–C) and their Newman projections are shown in Figure 4. Included in this figure are the values of the torsion angle θ_4 = O-(21)–C(2)–C(3)–O(31) (cf. Figure 1) defining the three staggered rotamers A–C. The fractional populations of rotamers A–C (cf. Figure 4) were calculated for all the compounds listed in Table I, and the results are summarized in Table II. From an inspection of this Table it is clear that there are two possible conformations about the C(2)–C(3) glycerol bond. This is true for both mono- (lyso) and diacyl (dialkyl) phospholipids differing in the nature of the polar group and regardless whether the phospholipids are present as monomers or aggregates. Furthermore, the nature of the solvent cannot be an important factor as noted before (Hauser et al., 1980a). Replacement of water of hydration by organic solvent has relatively little effect on the rotamer population. The two preferred conformations are rotamer A (Figure 4) characterized by torsion angles θ_3/θ_4 = ap/sc and rotamer B with torsion angles θ_3/θ_4 = +sc/–sc. The conformation of rotamer A is the one that is found in the majority of single-crystal structures of phospholipids, diglycerides, and related compounds (cf. Table III). Rotamer B is also represented in single-crystal structures of phospholipids though to a lesser extent. In monomeric solutions or micellar dispersions in which the phospholipid is liquid-crystalline, rotamer A is also the dominant conformation; the population ratio rotamer A/B varies between 1 and 2 for all compounds listed in Table II.

Both rotamers A and B (see Figure 4) are synclinal (gauche) rotamers, indicating that the two oxygens on carbon atoms C(2) and C(3) to which the hydrocarbon chains are attached adopt the synclinal (gauche) conformation, i.e., θ_4 = ±sc, respectively. In contrast, in rotamer C (Figure 4) these two oxygens and therefore θ_4 are antiperiplanar. In rotamers A and B the parallel alignment of the two hydrocarbon chains is readily accomplished while in rotamer C with θ_4 = ap parallel chain stacking is difficult to envisage. Therefore, rotamer C is energetically unfavorable. Indeed, rotamer C is practically absent in micelles of diacyl phospholipids (Table II). At concentrations <CMC where these phospholipids are present as monomers, rotamer C is populated though its contribution is usually less than 10%. An exception is di-hexyl-PC which has a slightly higher population of rotamer C (10–15%, Table II).

Table II: The Three Staggered Conformations of Minimum Free Energy (Rotamers A–C, Figure 4) about the C(2)–C(3) Glycerol Bond of Different Phospholipids[a]

compound	vicinal spin coupling const (Hz)		rotamer population (%)			exptl conditions
	$^3J_{AX(AC)}$	$^3J_{BX(BC)}$	A	B	C	
2,3-dihexanoyl-D-glycero-1-phosphochline	7.6	2.4	62	38	1	in ^2H_2O, >CMC
	6.8	3.0	52	42	6	in ^2H_2O, <CMC
2,3-dihexanoyl-D-glycero-1-phosphoethanolamine	7.7	2.3	63	37	1	in ^2H_2O, pH 6.0, >CMC
	7.9	1.8	68	32	0	in ^2H_2O, pH 9.3, >CMC
	7.2	3.1	55	38	7.5	in ^2H_2O, pH 6.0, <CMC
2,3-di-*O*-hexyl-D-glycero-1-phosphocholine	6.5	3.0	49	44	6.5	in ^2H_2O, >CMC
	5.6–6.5	3.3–3.8	38–48	42–47	10–15	in ^2H_2O, <CMC
2,3-dihexanoyl-D-glycero-1-phospho-L-serine ammonium salt	7.6	2.4	62	38	1	in ^2H_2O, >CMC
	7.1	3.0	55	39	6.5	in ^2H_2O, <CMC
2,3-dipalmitoyl-DL-glycero-1-phosphocholine	6.9	3.1	52	40	7.5	in $C^2HCl_3/C^2H_3O^2H$, 2:1 (v/v)
2,3-dipalmitoyl-DL-glycero-1-phosphoethanolamine	6.9	3.4	51	38	11	in $C^2HCl_3/C^2H_3O^2H$, 2:1 (v/v)
2,3-dipalmitoyl-D-glycero-1-phospho-L-serine	7.2	3.0	55	38	6.5	in $C^2HCl_3/C^2H_3O^2H$, 4:3 (v/v)
egg lysophosphatidylcholine	6.5	3.4	48	41	11	in ^2H_2O, >CMC
3-myristoyl-D-glycero-1-phosphocholine	6.7	4.1	46	36	18	in ^2H_2O, >CMC
3-lauroyl-D-glycerol 1-phosphate disodium salt	6.7	4.2	45	36	19	in ^2H_2O, >CMC
3-lauroylpropanediol-1-phosphocholine	6.73	6.61	36	32	32	in ^2H_2O, >CMC
	6.47	6.47	33	33	33	in $C^2H_3O^2H$

[a] Rotamer populations were calculated from the observed vicinal spin coupling constants as described previously (Hauser et al., 1978) and are expressed in percent.

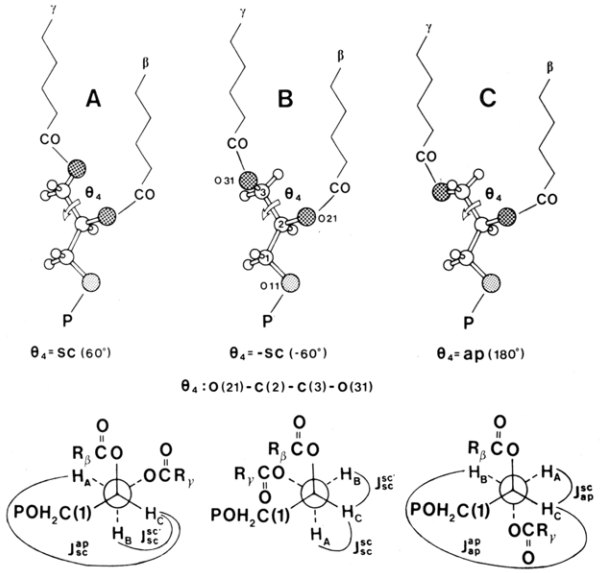

FIGURE 4: The three staggered conformations A–C of the glycerol C(2)–C(3) bond of phospholipids. For atom numbering and notation for torsion angles see Figure 1. Conformations A–C are characterized by two related torsion angles, θ_3 = C(1)–C(2)–C(3)–O(31) and θ_4 = O(21)–C(2)–C(3)–O(31). The torsion angle θ_4, which determines the relative orientation of the two ester or ether oxygen atoms O(21) and O(31) (hatched) on glycerol C(2) and C(3) is ±synclinal in rotamers A and B, respectively. These two conformations will be referred to as the synclinal or "gauche" rotamers. In contrast, in rotamer C, θ_4 is antiperiplanar and is therefore referred to as the antiperiplanar rotamer. The conformational notation used here is that of Klyne and Prelog [1960; see also Hauser et al. (1981)]. The ball and stick models of rotamers A–C are drawn with O(11) (stippled) downward to which the head group is attached. The asymmetric glycerol carbon C(2) atom has the natural D configuration. Newman projections of the three staggered conformations A–C are included at the bottom. Also included are the component vicinal ^1H spin coupling constants J. The subscript of J denotes the conformation of the e O(21)–C(2)–C(3)–O(31) bond (=θ_4), the superscript denotes the orientation of the spin-coupled protons. For instance, the component vicinal coupling constant between protons H_A and H_C in rotamer A would be J^{ap}_{sc} because rotamer A is synclinal (θ_4 = synclinal) and the torsion angle between these two protons H_C–C(2)–C(3)–H_A is antiperiplanar. The values for the component vicinal ^1H spin coupling constants used in this study are as follows: J^{sc}_{ap} = 5.8 Hz, J^{ap}_{ap} = 11.7 Hz (Abraham & Gatti, 1969), J^{ap}_{sc} = 12.0 Hz, J^{sc}_{sc} = 0.45 Hz, and $J^{sc/}_{sc}$ = 2.4 Hz (Gatti et al., 1967).

The predominant conformations below CMC are still those of rotamers A and B (Table II), indicating that the parallel alignment of the two hydrocarbon chains is preserved even in the monomeric state. Apparently, intramolecular chain stacking is the principle governing the conformation about the C(2)–C(3) glycerol bond of phospholipids: it is energetically favorable because it probably optimizes both van der Waals and hydrophobic interactions. Comparing the rotamer populations of short-chain diacyl phospholipids in H_2O above and below the CMC, it appears that in the monomeric state the populations of rotamers B and C are increased at the expense of rotamer A. It is somewhat surprising that rotamers A and B are also predominant in lysophospholipids though the population of rotamer C is increased in this class of lipids, amounting to 10–20% (Table II). Since lysophospholipids have a single hydrocarbon chain in the glycerol C(3) position and a free OH group on glycerol C(2), intramolecular chain stacking cannot come into play. No definite interpretation can be offered at present. However, the comparison of the lysophospholipid data with the result obtained with 3-lauroylpropanediol-1-phosphocholine (last entry of Table II) can possibly shed light on this question. In both water and methanol the latter compound does not exhibit a conformational preference about the C(2)–C(3) bond of its 1,3-propanediol moiety. All three staggered rotamers are equally populated, and there is probably free rotation about the C(2)–C(3) bond. This indicates that in 3-lauroylpropanediol-1-phosphocholine there are no stringent structural requirements governing the packing of these molecules in micelles and ruling out certain rotamers about the C(2)–C(3) bond. Since the structural difference between lysoglycerophospholipids and 1,3-propanediol-containing lipids is the free OH group on C(2), a stabilizing role may be attributed to this OH group. The preferred conformations of rotamers A and B in lysophospholipids may be stabilized by intra- and/or intermolecular hydrogen bonding involving the free OH group on glycerol C(2).

As mentioned briefly above, the exchange rate between rotamers A and B is fast on the NMR time scale. This means that the diacyl phospholipids and the lysophospholipids listed in Table II switch rapidly between essentially two conformations about the C(2)–C(3) glycerol bond, one given by θ_3/θ_4

Table III: Torsion Angles Defining the Polar Group Conformation and Parallel Alignment of the Hydrocarbon Chains in Phospholipid Single-Crystal Structures

phospholipid	rotamer C(2)–C(3) glycerol	orientation of glycerol moiety	α_1	α_2	α_3	α_4	α_5	θ_1/θ_2	θ_3/θ_4	β_1	β_2	β_3	β_4	γ_1	γ_2	γ_3	γ_4	ref
DMPC1	A_γ	⊥	163	62	68	143	−64	58/177	−178/63	82	172	−81	45	−177	168	−173	178	*a*
DLPA	A_β	parallel	−151					64/−171	−171/62	83	174	164	173	−173	−176	−77	70	*b*
$DLPEM_2$	B_γ	45°	179	65	54	144	−94	176/−66	54/−60	148	173	−57	176	129	−167	166	175	*c*
DMPG1	B_β	45°	−146	−76	−86	143	180	151/−78	64/−63	159	178	177	−176	164	−170	110	−57	*d*
GPC2	C		172	64	65	140	−75	−63/61	−69/169									*e*
GPE	C		−174	−81	−81	164	55	166/−89	−67/180									*f*

[a] Pearson & Pascher, 1979. [b] Pascher and Sundell, unpublished results. [c] Pascher & Sundell, 1986. [d] Pascher et al., 1987. [e] Abrahamsson & Pascher, 1986. [f] de Titta & Craven, 1971.

= ap/+sc (rotamer A) and the second one given by θ_3/θ_4 = +sc/−sc (rotamer B). This interconversion between the two dominant rotamers A and B is depicted in Figure 6 (see discussion below).

From the NMR data presented it can be concluded that the motionally averaged conformation of the glycerol backbone is similar in different phospholipids. Seelig and co-workers arrived at the same conclusion from deuterium NMR applied to liquid-crystalline bilayers of different phospholipids selectively deuteriated in the glycerol backbone (Browning & Seelig, 1980; Gally et al., 1981; Ghosh & Seelig, 1982; Borle & Seelig, 1983; Allegrini et al., 1984). Various spectroscopic methods have been applied to the problem of the average conformation and the dynamics of the glycerol group in phospholipid aggregates, particularly in the smectic (lamellar) phase. The results are inconclusive, and their interpretation varies from a rigid glycerol backbone with a single conformation about the C(2)–C(3) bond to two or more conformations that are in rapid equilibrium. For instance, deuterium NMR results obtained with phosphatidylethanolamines and phosphatidylglycerols derived from *Escherichia coli* and selectively deuteriated in the glycerol group were interpreted in terms of at least two conformations about the glycerol C(2)–C(3) bond (Gally et al., 1981) consistent with the model presented here. The two deuterons in position C(3) were found to be magnetically inequivalent. A 2-fold jump about the C(2)–C(3) bond between rotamer A and B was invoked in order to account for the observed deuterium quadrupole splittings. Similar deuterium NMR results were reported by Blume et al. (1982), who investigated bilayers of dipalmitoylphosphatidylethanolamine selectively deuteriated in the glycerol C(2) position both above and below the transition temperature. The gel-phase spectra were interpreted in terms of a single population of rotamer B. Upon passage through the transition the deuterium quadrupole splitting decreased by a factor of 4. This dramatic narrowing was attributed to a conformational change in the glycerol backbone. These authors suggested that the observed quadrupole splitting in liquid-crystalline dipalmitoylphosphatidylethanolamine bilayers could be explained in terms of a single conformation about the C(2)–C(3) glycerol bond which would have to be different from both rotamers A and B. Alternatively, a rapid equilibrium among two or more weighted conformations about the C(2)–C(3) bond could also account for the observed quadrupole splittings. In contrast, Strenk et al. (1985) interpreted deuterium NMR results obtained with liquid-crystalline bilayers of dimyristoylphosphatidylcholine selectively deuteriated in the glycerol group in terms of a rigid glycerol C(2)–C(3) bond. The single conformation about this bond was concluded to be identical with that found in the single-crystal structure of dilauroylphosphatidylethanolamine (Strenk et al., 1985). This interpretation is at variance with the deuterium NMR results of Gally et al. (1981) and Blume et al. (1982). Clearly, more work on phospholipid bilayers is required in order to unravel the existing discrepancy in the literature. At this point it is worth noting that the coexistence of the two rotamers A and B is also consistent with potential energy calculations of McAlister et al. (1973). Two conformations about the C(2)–C(3) glycerol bond identical with those of rotamers A and B were identified as minimum-energy conformations of approximately the same value.

X-ray Single-Crystal Structures. The torsion angles defining the polar group conformation in the single-crystal structures of some diacyl phospholipids and related compounds are summarized in Table III. Regarding the conformation about the C(2)–C(3) glycerol bond, the existing single-crystal structures of phospholipids and phospholipid constituent molecules can be arranged into three classes corresponding to rotamers A–C (see second column, Table III). The first class, characterized by torsion angles θ_3/θ_4 = ap/sc and consistent with rotamer A (Figures 4 and 5), is represented by DMPC (Pearson & Pascher, 1979) and DLPA (Pascher and Sundell, unpublished results), the second class, with θ_3/θ_4 = +sc/−sc consistent with rotamer B, is represented by $DLPEM_2$ (Pascher & Sundell, 1986) and DMPG (Pascher et al., 1987), and the third one, with θ_3/θ_4 = −sc/ap consistent with rotamer C, is represented by small molecules such as GPC (Abrahamsson & Pascher, 1966) and GPE (de Titta & Craven, 1971). As mentioned above, θ_4 = ±sc allows readily the parallel alignment of the hydrocarbon chains. The majority of compounds whose single-crystal structure has been solved belong to class 1: in addition to DMPC and DLPA (listed in Table III), 2,3-dilauroyl-DL-glycero-1-phosphoethanolamine (Hitchcock et al., 1974; Elder et al., 1977), sodium 2,3-dimyristoyl-D-glycerol 1-phosphate (Harlos et al., 1984), 3-octadecyl-2-methyl-D-glycero-1-phosphocholine (Pascher et al., 1986), 3-palmitoyl-DL-glycero-1-phosphoethanolamine (Pascher et al., 1981a), the disodium salt of 3-lauroyl-DL-glycerol 1-phosphate (Pascher & Sundell, 1985), and 2,3-dilauroyl-D-glycerol (Pascher et al., 1981b). Fewer molecules are found in class 2 with θ_3 = +sc (range 45–69°) and θ_4 = −sc (range −51 to −63°). In addition to $DLPEM_2$ (Pascher & Sundell, 1986) and DMPG (Pascher et al., 1987) listed in Table III there is a second conformational isomer in the single-crystal structure of DMPG (Pascher et al., 1987), as well as 2,3-bis(11-bromoundecanoyl)-DL-glycero-*p*-toluenesulfonate (Watts et al., 1972) and the $CdCl_2$ complex of GPC (Sundaralingam & Jensen, 1965). As expected, only compounds without hydrocarbon chains are found in class 3 having the conformation of rotamer C (θ_3/θ_4 = −sc/ap). As mentioned above, this rotamer with θ_4 = ap and hence the two oxygen atoms on C(2) and C(3) in an antiperiplanar arrangement is difficult to reconcile with the parallel alignment of the hydrocarbon chains and would therefore be energetically unfa-

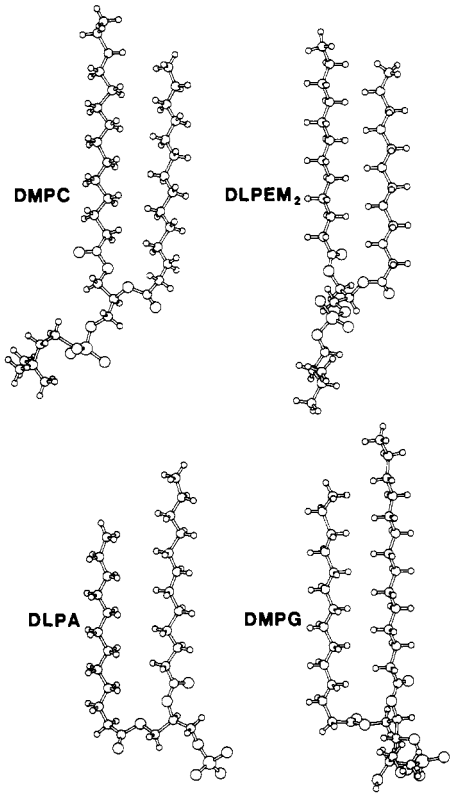

FIGURE 5: Molecular conformation of four single-crystal structures, DMPC (Pearson & Pascher, 1979), DLPEM$_2$ (Pascher & Sundell, 1986), DLPA (Pascher and Sundell, unpublished results), and DMPG (Pascher et al., 1987). The four selected single-crystal structures represent possible conformations about the C(2)–C(3) glycerol bond and related to these conformations possible chain stacking modes. DMPC and DLPA (left-hand side) are representative of rotamer A, and DLPEM$_2$ and DMPG (right-hand side) represent rotamer B. The two representative structures of rotamer A, DMPC and DLPA, differ in both the chain stacking and the orientation of the glycerol group with respect to the bilayer plane. The two representatives of rotamer B, DLPEM$_2$ and DMPG, differ only in the stacking of the fatty acyl chains; their glycerol groups have similar orientations. Structural details are discussed in the text (see also Figure 6).

vorable for compounds with two acyl or alkyl chains.

The single-crystal structures of the diacyl phospholipids in Table III were selected from all existing single-crystal structures to demonstrate the possible minimum free energy conformations about the C(2)–C(3) glycerol bond and possible ways of chain stacking associated with these different conformations. It is clear from Table III that there are not only two types of conformations about the C(2)–C(3) glycerol bond represented by rotamers A and B, respectively, but there are also two types or subgroups of rotamers A and B. These two subgroups differ in the orientation of the glycerol backbone and related to it in their chain stacking (Table III). Greek subscripts are used to identify the two different subgroups of rotamers A and B (see second column, Table III). The subscript β (γ) indicates that the β- (γ-) chain is straight, extending in an almost perfect continuous zigzag from the glycerol backbone. The other hydrocarbon chain is initially oriented layer-parallel and undergoes an approximately 90° bend at its second C atom. Representative single-crystal structures of the two subgroups of rotamers A and B, respectively, are depicted in Figure 5. The two subgroups clearly differ in chain stacking and possibly in the orientation of the glycerol group (see third column, Table III). The two subgroups of rotamer A are represented by DMPC and DLPA (Figure 5, Table III). In DMPC the γ-chain is oriented approximately perpendicular to the bilayer plane while the β-chain has initially a layer-parallel orientation (Figure 5). At the second carbon atom C(22) of the β-chain there is a 90° bend so that from C(22) onward this chain becomes parallel to the γ-chain. The single-crystal structure of DMPC is therefore designated A_γ to indicate that the γ-chain forms a straight zigzag arrangement. This is contrasted by the packing of the two hydrocarbon chains in DLPA representing the second subgroup of rotamer A. The β-chain extends straight upward from glycerol atom C(2) while the γ-chain is initially oriented layer-parallel. At carbon atom C(32) the γ-chain makes a 90° bend (Figure 5, bottom left). The structure of DLPA is therefore designated as A_β (see second column, Table III). DMPC and DLPA also differ in the orientation of the glycerol group with respect to the bilayer plane (see third column in Table III). In DMPC the glycerol group is oriented approximately perpendicular to the bilayer plane, forming a continuous antiperiplanar zigzag with the γ-acyl (alkyl) chain. In contrast, the orientation of the glycerol group of DLPA is parallel to the bilayer plane (Figure 5, bottom left) despite the fact that torsion angles θ_3 and θ_4 are practically identical with those of DMPC (Table III).

The two subgroups of rotamer B are represented by the single-crystal structures of DLPEM$_2$ and DMPG (Table III, Figure 5, right panel). In the single-crystal structure of DLPEM$_2$ (Figure 5) the γ-chain extends almost straight from glycerol C(3) with a minor twist about the C(3)–O(31) bond (γ_1 = 129°, see Table III). The β-chain makes a 60° bend at carbon atom C(22) due to a –sc twist (β_3 = –57°) about the C(21)–C(22) bond. In this way the parallel alignment of the two hydrocarbon chains is accomplished (Figure 5). In contrast, rotamer B (θ_3/θ_4 = sc/–sc) of DMPG (Table III) exhibits a stacking mode different from DLPEM$_2$ (compare Figure 5, top and bottom of right panel). The glycerol group is rotated in such a way that oxygens O(21) and O(31) are turned into an approximately layer-normal and a layer-parallel position, respectively. Due to this orientation the β-chain extends straight while the γ-chain makes a 90° bend at carbon atom C(32). However, the orientation of the glycerol group with respect to the bilayer plane is similar to that of DLPEM$_2$. In both cases the glycerol groups are inclined by approximately 45°.

Amalgamating the information derived from NMR (Table II) with the information summarized in Table III, some conclusions are obvious. The single-crystal structures lend support to the notion that the two preferred conformations about the glycerol C(2)–C(3) bond (rotamers A and B) derived from the NMR data are indeed minimum free energy conformations of the diacylglycerol part of lipids. The NMR data also indicate that there is rapid interconversion on the NMR time scale between the two rotamers A and B in the liquid-crystalline state of phospholipids.

The structure and dynamics of the glycerol backbone as the central part of phospholipid molecules is of paramount importance for understanding the structural role of these molecules. It is still an unsolved problem. On the basis of existing NMR data and the single-crystal structures presented in Table III and Figure 5, a hypothesis can be put forward pertaining to the question of the structure and dynamics of the glycerol C(2)–C(3) group in phospholipids. We propose that in liquid-crystalline phospholipid bilayers the molecules undergo conformational changes between rotamers A and B (see Figure 6). These transitions involve primarily a change in torsion angles θ_3 and θ_4. Furthermore, they must involved conformational changes in the two fatty acyl chains if the parallel alignment of the two fatty acyl chains is to be maintained. The

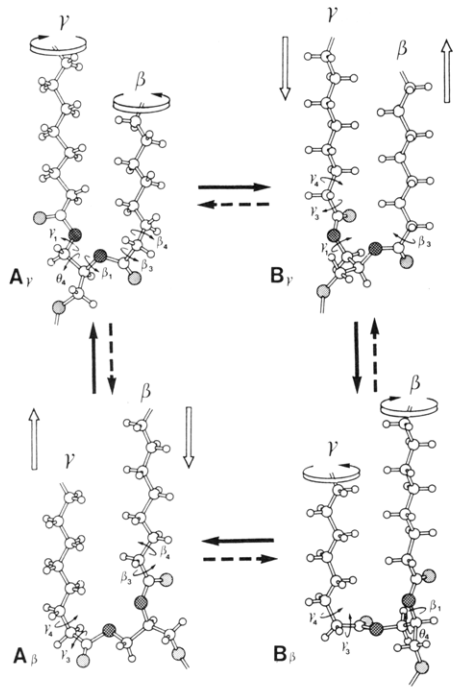

FIGURE 6: The four single-crystal structures of Figure 5 are shown: DMPC (top left), DLPA (bottom left), $DLPEM_2$ (top right), and DMPG (bottom right) together with their designations A_γ, A_β, B_γ, and B_β, respectively. The letters refer to rotamers A and B differing in the conformation of the C(2)–C(3) glycerol bond: rotamer A, θ_3 = ap, θ_4 = sc; rotamer B, θ_3 = sc, θ_4 = –sc. The subscript refers to the chain stacking, β indicating that the β-chain extends as a straight zigzag chain from glycerol C(2) and γ indicating that this is the case for the γ-chain attached to glycerol C(3). The ester oxygens attached to the glycerol C(2)–C(3) bond are emphasized by heavy shading, and all other oxygens are shaded. Structures A_γ and A_β also differ in the orientation of the glycerol group with respect to the bilayer plane, this group being oriented approximately perpendicular and parallel, respectively (see also Table III). The orientation of the glycerol group is defined by the angle between the vector connecting glycerol C(1) and glycerol C(3) and the plane of the bilayer. While in structures B_γ and B_β the chain stacking is different, resembling that observed in A_γ and A_β, respectively, the orientation of the glycerol group is in both cases about 45°. Also indicated in this diagram are transitions between the four different single-crystal structures and the conformational changes associated with these transitions. The transitions indicated by horizontal heavy arrows involve primarily conformational changes between rotamers A and B or vice versa involving torsion angles θ_3/θ_4. The transitions indicated by vertical heavy arrows indicate primarily changes in chain stacking whereby the conformation about the C(2)–C(3) bond (torsion angles θ_3/θ_4) remains unchanged. The dashed (heavy) arrows serve to indicate the reversible character of these transitions. The small arrows associated with torsion angles β_1, β_3, β_4, and γ_1 in structure A_γ indicate that these torsion angles undergo conformational changes in going from structure A_γ to B_γ in order to maintain the parallel alignment of the two hydrocarbon chains. The light arrows on top of the two hydrocarbon chains of A_γ indicate the overall rotation of the hydrocarbon chains in going from structure A_γ to B_γ. Changes in the orientation of the glycerol group may or may not be recognized from an inspection of the figure. Whether such a change is involved can be derived from Table III (third column). The sum of the changes involved in the transition from structure B_γ to B_β is indicated by various arrows associated with the B_γ structure; similarly, changes involved in going from B_β to A_β and A_β to A_γ are indicated by arrows in B_β and A_β, respectively. The light arrows at the hydrocarbon chain ends of structure B_γ indicate that on going from B_γ to B_β the chain stacking mode is changed, involving stretching of the β-chain and shortening of the γ-chain of B_γ. Shortening of the γ-chain is produced by introducing an approximately 90° kink at carbon atom C(2).

sum of the conformational changes involved in the transition from rotamer A to rotamer B occur with rates that are fast on the NMR time scale. The four single-crystal structures presented in Figures 5 and 6 (cf. Table III) are used to get an idea about the conformational changes in the two fatty acyl chains associated with the transition between rotamers A and B or vice versa. The motionally averaged conformation of the C(2)–C(3) glycerol bond of phospholipids in liquid-crystalline bilayers may be an average of the four minimum-energy conformations presented in Figure 6. Their weighting factor which is presently unknown would reflect the relative probability of the four conformations. It should be stressed that these four structures (cf. Figures 5 and 6) not only differ in torsion angles θ_3/θ_4 characterizing the conformation of the glycerol C(2)–C(3) bond but also in chain stacking and, related to it, in the orientation of the glycerol backbone. The details of the conformational changes involved in transitions between the four stable conformations of the C(2)–C(3) glycerol bond may be read off Figure 6. These may be regarded as minimum changes. The conformational changes accompanying the transition from structure A_γ to B_γ are indicated by small arrows in rotamer A_γ. Torsion angles undergoing changes are inserted in A_γ. The transition from rotamer A_γ to B_γ involves primarily a change in θ_3 from ap to +sc and θ_4 from +sc to –sc. This change in torsion angles θ_3/θ_4 is produced by an anticlockwise rotation by 120° of the entire γ-chain about the C(2)–C(3) glycerol bond of rotamer A, as indicated by the small arrow about the C(2)–C(3) bond of structure A_γ (Figure 6). As a result of the 120° anticlockwise rotation about the C(2)–C(3) bond the parallel alignment of the two hydrocarbon chains is lost. In order to realign the two hydrocarbon chains, there is a significant change in γ_1 and a simultaneous clockwise rotation of the β-chain with changes in torsion angles β_1, β_3, and β_4 (marked by small arrows in the structure A_γ of Figure 6) as well as a change in the orientation of the glycerol group. This group changes from an approximate perpendicular orientation with respect to the bilayer plane to one that is tilted by about 45° (cf. A_γ and B_γ in Figure 6 and see also Table III). The reverse transition from rotamer B_β to A_β involves primarily the change in θ_3/θ_4 = +sc/–sc to θ_3/θ_4 = ap/+sc. This conformational change of the C(2)–C(3) glycerol group necessitates conformational changes in both fatty acyl chains in order to maintain their parallel alignment. Significant changes occur in torsion angles β_1, γ_3, and γ_4 as indicated in B_β (Figure 6, Table III). Transitions indicated by heavy vertical arrows in Figure 6, i.e., from A_β to A_γ and B_γ to B_γ, do not involve conformational changes in the C(2)–C(3) glycerol bond. These two transitions effect primarily changes in the chain stacking and related to it changes in the orientation of the glycerol group, as, for instance, in the A_β to A_γ transition. The orientation of the glycerol group changes from parallel to the bilayer plane in the A_β structure of DLPA to perpendicular in the structure of A_γ of DMPC (Figure 6). The transition from A_β to A_γ involves an axial displacement of the two hydrocarbon chains by four methylene groups (see light arrows at the end of the hydrocarbon chains of A_β, Figure 6). By this the γ-chain straightens out while the β-chain attains a 90° kink at carbon atom C(22). Such a change in the chain stacking involves significant conformational changes in torsion angles β_3, β_4, γ_3, and γ_4, as indicated by the small arrows inserted in the A_β structure (Figure 6, Table III).

A similar axial displacement of the two hydrocarbon chains by four methylene groups occurs in going from B_γ to B_β (right panel, Figure 6). In this case the β-chain straightens out and the γ-chain attains a 90° kink at carbon atom C(32). This change in chain stacking is accompanied by significant conformational changes in torsion angles β_3, γ_1, γ_3, and γ_4, as indicated in the B_γ structure (Figure 6 and Table III).

In summary, we have made use of existing single-crystal structures of phospholipids to propose possible conformational changes in the hydrocarbon chains and/or orientation of the glycerol group that accompanies the transition from rotamer A to B. These conformational changes in the β- and γ-chain represent minimal changes required to maintain the parallel alignment of the hydrocarbon chains. The conformational changes taking place in liquid-crystalline phases of phospholipid bilayers and micelles may be more complex involving a larger number of bonds. The ideas developed from NMR results and X-ray crystallography for the motion of the diacylglycerol portion of phospholipids in the liquid-crystalline state ought to be subjected to experimental test. Clearly, the NMR method used in this work cannot detect changes in the orientation of the glycerol group, for vicinial spin coupling is only sensitive to changes in torsion angles. Therefore, other methods that are sensitive to changes in orientation are required, for instance, deuterium NMR and IR spectroscopy. In case of deuterium NMR the line shape reflects the motional averaging of the field gradient tensor, and from the spectral line shape and the knowledge of the orientation of the field gradient tensor conclusions can be drawn regarding the motional averaging processes. IR spectroscopy is probably a useful and important complementary method. Since its time scale is on the order of 10^{-10} s, which is much shorter than that of NMR methods, IR can possibly provide information as to the number of different C(2)–C(3) conformations (e.g., A_γ, A_β, ...) contributing to the averaging observed by NMR techniques. The rate of transitions between different C(2)–C(3) conformations is certainly smaller than 10^{10} s^{-1}, and hence different conformers can be expected to give rise to separate IR bands. The carbonyl (C=O) stretching modes are suitable candidates for testing this assumption. Currently, deuterium NMR work with different phospholipids selectively deuteriated in the glycerol group is carried out in our laboratory. The purpose of this work is to subject the hypothesis developed in this study to experimental test. The final goal is the elucidation of the structure and dynamics of the diacylglycerol part of phospholipids in bilayers and membranes.

ACKNOWLEDGMENTS

We are indebted to Max Lundmark for skillful technical assistance.

Registry No. DHPC, 34506-67-7; DHPE, 96893-06-0; DHPS, 84773-42-2; dihexyl-PC, 79645-39-9; DMPC, 18194-24-6; DLPA, 55332-91-7; $DLPEM_2$, 101218-35-3; DMPG, 61361-72-6; GPC, 28319-77-9; GPE, 33049-08-0; 2,3-dipalmitoyl-DL-glycero-1-phosphocholine, 2797-68-4; 2,3-dipalmitoyl-DL-glycero-1-phosphoethanolamine, 5681-36-7; 2,3-dipalmitoyl-D-glycero-1-phospho-L-serine, 40290-42-4; 2,3-dipalmitoyl-D-glycero-1-phosphate, 7091-44-3; 3-myristoyl-D-glycerol 1-phosphate, 20559-16-4; 3-lauroylpropanediol-1-phosphocholine, 25800-40-2; 3-lauroyl-D-glycerol 1-phosphate, 20559-18-6.

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