Wohlgemuth, R., et al.: Bilayers of phosphatidylglycerol. A deuterium and phosphorus nuclear magnetic resonance study of the head-group region. Biochemistry 19, 3315

ARTICLE *in* BIOCHEMISTRY · AUGUST 1980

Impact Factor: 3.02 · DOI: 10.1021/bi00555a033 · Source: PubMed

3 AUTHORS, INCLUDING:

Roland Wohlgemuth
Sigma-Aldrich, Member of Merck Group
143 PUBLICATIONS **2,001** CITATIONS
SEE PROFILE

Joachim Seelig
University of Basel
289 PUBLICATIONS **17,857** CITATIONS
SEE PROFILE

Bilayers of Phosphatidylglycerol. A Deuterium and Phosphorus Nuclear Magnetic Resonance Study of the Head-Group Region†

Roland Wohlgemuth, Nada Waespe-Šarčević, and Joachim Seelig*

ABSTRACT: The structural properties of the phosphoglycerol polar head group in bilayers of 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol have been studied with deuterium and phosphorus-31 nuclear magnetic resonance. For this purpose, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol was deuterated chemically or biosynthetically at all three segments of the glycerol head group. Both the D and the L configurations of the glycerol head group have been synthesized, and the correct stereochemical configuration of the polar group was verified by an enzymatic assay, as direct measurement of the optical rotation was insufficiently sensitive to distinguish between these diastereomers. The phosphatidylglycerol sodium salts were dispersed in excess buffer (0.1 M NaCl, pH 7.0), and the bilayer properties were characterized by small-angle X-ray diffraction and differential scanning calorimetry. The deuterium quadrupole splittings, $\Delta\nu_Q$, and the phosphorus-31 chemical shielding anisotropy, $\Delta\sigma$, were measured as a function of temperature in the range 20–60 °C, yielding the following results. (1) Well-resolved deuterium signals were obtained for all head-group segments and could be assigned unambiguously. (2) Almost identical spectra were obtained for 1,2-dipalmitoyl-*sn*-glycero-3-phospho-1′-glycerol (natural L,D configuration) and 1,2-dipalmitoyl-*sn*-glycero-3-phospho-3′-glycerol (L,L configuration), suggesting very similar head-group motions and orientations for both diastereomers. (3) The spatial anisotropy of motion and the segmental fluctuations of the negatively charged phosphoglycerol are similar to those of the zwitterionic phosphocholine and phosphoethanolamine head groups but differ distinctly from those of phosphoserine which also carries a net negative charge. (4) The motional inequivalence of geminal deuterons in 1,2-dipalmitoyl-*sn*-glycero-3-phospho-3′-glycerol was demonstrated by synthesis of a stereospecifically monolabeled analogue.

Phosphatidylglycerol is one of the major phospholipid classes and has been found to occur in higher plants, algae, bacteria and to a lesser extent in animal tissue (cf. Bonsen et al., 1966; Sacrē & Tocanne, 1977; Rottem & Markowitz, 1979). Because of its negatively charged head group [pK = 3–5.5, depending on the ionic composition; Sacrē & Tocanne (1977), Watts et al. (1978)], the lipid is most probably involved in the interaction of membranes with ions and proteins. Physicochemical studies have been carried out to explore the properties of bilayers composed of either pure phosphatidylglycerol or mixtures of phosphatidylglycerol with other types of phospholipids. Most of these studies have been concerned with the thermodynamic phase behavior as detected by fluorescence polarization, permeability measurements, differential scanning calorimetry, and spin-label electron-spin resonance (Papahadjopoulos et al., 1973, 1975; Verkleij et al., 1974; Jacobson & Papahadjopoulos, 1975; Ververgaert et al., 1975; van Dijck et al., 1975, 1978; Papahadjopoulos, 1977; Findlay & Barton, 1978; Watts et al., 1978). In addition, the interaction of synthetic phosphatidylglycerol and some of its derivatives with monovalent and divalent ions has been investigated extensively by the monolayer technique (Tocanne et al., 1974a,b; Sacrē & Tocanne, 1977; El Mashak & Tocanne, 1979). A detailed X-ray analysis of aqueous dispersions of 1,2-dipalmitoyl-*sn*-glycero-3-phospho-*rac*-glycerol has also been published (Ranck et al., 1977).

In the present report, we describe results related to the structural and motional properties of the phosphoglycerol head-group region in bilayers of 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol (DPPG).[1] By analogy with similar studies on phosphatidylcholine (Gally et al., 1975; Seelig et al., 1977), phosphatidylethanolamine (Seelig & Gally, 1976), and phosphatidylserine (Browning & Seelig, 1980), we have selectively deuterated the various segments of the glycerol head-group moiety and have measured the deuterium quadrupole splittings and phosphorus-31 chemical shift anisotropy of the lipid bilayer in excess buffer as a function of temperature. A complication in the synthesis of the deuterated phosphatidylglycerol arises from the fact that natural phosphatidylglycerol has the L,D configuration in the two glycerol moieties (3-*sn*-phosphatidyl-1′-*sn*-glycerol) (cf. Bonsen et al., 1966). However, in most model studies, the lipid has been prepared by transphosphatidylation of phosphatidylcholine with phospholipase D in the presence of glycerol which leads to a mixture of diastereomers, i.e., 3-*sn*-phosphatidyl-1′-*sn*-glycerol and 3-*sn*-phosphatidyl-3′-*sn*-glycerol (Joutti & Renkonen, 1976). Since the similarity or dissimilarity of the physical properties of the two diastereomers was unknown, some of the lipids employed in this work have been prepared by stereospecific chemical synthesis leading to either 3,1′- or 3,3′-phosphatidylglycerol and allowing a comparison of the two diastereomers.

Materials and Methods

Synthesis of 1,2-Dipalmitoyl-sn-glycero-3-phosphoglycerol. Various methods for the chemical synthesis of saturated and unsaturated phosphatidylglycerols have been discussed in the literature (Baer & Buchnea, 1958; Kiyasu et al., 1963; Saunders & Schwarz, 1966; Bonsen et al., 1966; Tocanne et al., 1974; Lammers & van Boom, 1977, 1979). The most convenient route for our purposes was the coupling of phos-

† From the Department of Biophysical Chemistry, Biocenter of the University of Basel, CH-4056 Basel, Switzerland. *Received November 30, 1979.* This work was supported by Swiss National Science Foundation Grant No. 3.409.78.

[1] Abbreviations used: NMR, nuclear magnetic resonance; TPS, 2,3,5-triisopropylbenzenesulfonyl chloride; Pipes, 1,4-piperazinediethanesulfonic acid; DPPG, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol; 3,1′-DPPG, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-1′-*sn*-glycerol; 3,3′-DPPG, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-3′-*sn*-glycerol; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; DMPS, 1,2-dimyristoyl-*sn*-glycero-3-phospho-L-serine.

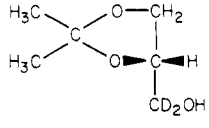
0006-2960/80/0419-3315$01.00/0

phatidic acid with isopropylideneglycerol using 2,4,6-triisopropylbenzenesulfonyl chloride (TPS) as the condensing agent (Aneja et al., 1970), followed by removal of the protecting group with boric acid in trimethyl borate (Mattson & Volpenheim, 1962; Bonsen et al., 1966).

Typically, 0.5 g of 1,2-dipalmitoyl-*sn*-glycero-3-phosphoric acid [prepared according to Baer (1951)], 0.55 g of TPS, and 0.5 g of 1,2-*O*-isopropylidene-*sn*-glycerol (D-acetone glycerol; cf. below) were dissolved in 10 mL of pyridine (freshly distilled over KOH). The mixture was heated to 50 °C, and the clear solution was stirred under dry nitrogen for 3 h. H_2O (0.5 mL) was added to destroy excess TPS, and the solution was stirred vigorously for 30 min. The solvents were removed at 40 °C under reduced pressure, and a small amount of toluene was added to facilitate the removal of the last traces of H_2O and pyridine. The remaining solids were extracted with ether, the ether suspension was filtered, and the clear solution was concentrated under reduced pressure. The product was purified by column chromatography [silica gel, 70–230 mesh; solvent, methylene chloride/methanol/water (65:25:4)], yielding 0.6 g of protected phosphatidylglycerol. The protecting group was removed as described by Bonsen et al. (1966), and the free phosphatidylglycerol was chromatographed over silica gel (70–230 mesh) with methylene chloride/methanol in ratios of (1) 400 mL, 85:15, (2) 400 mL, 75:25, and (3) 200 mL, 65:25, and with 200 mL of chloroform/methanol/water, 65:25:4 at room temperature. The product was finally eluted at 40–45 °C with about 800 mL of chloroform/methanol/water (65:25:4) to yield about 0.2–0.3 g of 1,2-dipalmitoyl-*sn*-glycero-3-phospho-3′-*sn*-glycerol (acid form). The sodium salt was formed by dissolving the free acid form of DPPG in chloroform/methanol and washing the organic phase with a saturated NaCl solution containing 0.1 M EDTA, pH 8.0.

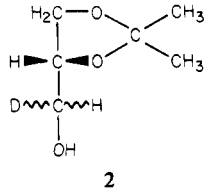
Synthesis of Deuterated 1,2- and 2,3-O-Isopropylidene-sn-glycerol. The essential precursor in the synthesis of head-group-deuterated DPPG was 1,2-*O*-isopropylidene-*sn*-glycerol (D-acetone glycerol) or 2,3-*O*-isopropylidene-*sn*-glycerol (L-acetone glycerol). The following compounds were synthesized by established methods.

1,2-O-Isopropylidene-sn-[3-$^2H_2$]glycerol (**1**). D-Mannitol was converted to the 1,2,5,6-diisopropylidene derivative, oxidized with lead tetraacetate to two molecules of isopropylidene-D-glyceraldehyde, oxidized further with $KMnO_4$ to the corresponding acid, esterified with CH_2N_2, and reduced with $LiAlD_4$ (Baer & Fischer, 1939a,b; Reichstein et al., 1935; Baer, 1952), leading to **1** [$[\alpha]^{20}_{546}$ +17.2° (neat)].



1

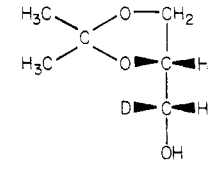
2,3-O-Isopropylidene-sn-[1-^2H]glycerol (**2**). L-Mannitol was prepared from L-arabinose (Baer & Fischer, 1939a,b) and converted to the 1,2,5,6-diisopropylidene derivative. Oxidation with sodium periodate followed by reduction with sodium borodeuteride (Lecocq & Ballou, 1964) resulted in **2** ($[\alpha]^{20}_{546}$ −18.2°, neat). A much simpler synthesis of L-acetone glycerol which avoids the rather tedious reaction sequence from L-arabinose to L-mannitol has been worked out by Lok et al. (1976) and has been employed successfully in later preparations of **2** in this laboratory.



2

1,2-O-Isopropylidene-(3R)-sn-[3-^2H]glycerol (**3**). It should be noted that the deuterium in **2** was not incorporated stereospecifically. Such a stereospecific incorporation can be achieved by enzymatic methods. Günther et al. (1973) have proposed an elegant procedure for the stereospecific introduction of deuterium at the C-1 atoms of primary alcohols such as ethanol, propanol, and butanol using an exchange reaction based on an enzyme-coupled recycling of two coenzymes. In the original procedure, yeast alcohol dehydrogenase was used for the catalyzed oxidation of the alcohols. However, for the preparation of 1,2-*O*-isopropylidene-(3*R*)-*sn*-[3-^{2}H]glycerol (**3**), the alcohol dehydrogenase from horse liver (HLADH) was employed since this enzyme is known to catalyze oxidoreduction of a much broader spectrum of substrates (Bentley, 1970).

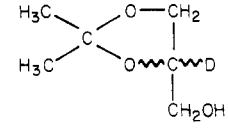
In 145 mL of deuterium oxide, 11.2 mmol of phosphate buffer (pH 8.0), 5.00 g (0.038 mol) of 1,2-isopropylidene-*sn*-glycerol, 30 mg of NAD^+, 50 mg of NADH, 50 mg of EDTA (disodium salt), 125 units of diaphorase from pig heart (Boehringer Mannheim; EC 1.6.4.3), 270 units of HLADH (Boehringer Mannheim; EC 1.1.1.1), and 100 mg of albumin were incubated for 72 h at 35 °C. The reaction mixture was extracted with chloroform, the extract dried (over Na_2SO_4), and the chloroform removed under reduced pressure. The oily residue was further purified by distillation to give 4.12 g (81.6%) of 1,2-*O*-isopropylidene-(3*R*)-*sn*-[3-^{2}H]glycerol (**3**):



3

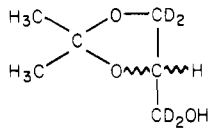
$[\alpha]^{25}_D$ +15.2° (neat); IR (film) 2140 cm^{-1} (C–D); ^{1}H NMR ($CDCl_3$) 4.30–3.40 (m, 4 H, glycerol backbone), 1.42 (s, 3 H, CH_3), 1.36 ppm (s, 3 H, CH_3). From selectively decoupled ^{1}H NMR spectra of derivatives of **3** as well as the corresponding nondeuterated compounds, it could be proven that one deuterium has been stereospecifically introduced at the C-3 of 1,2-*O*-isopropylidene-*sn*-glycerol. Compound **3** is thus equivalent to **1** with the exception that only one hydrogen at the *sn*-3 position has been replaced (stereospecifically) by deuterium.

rac-Isopropylidene[2-^2H]glycerol (**4**). Dihydroxyacetone was reduced with $NaBD_4$ in H_2O, and salts were removed by mixed-bed ion-exchange chromatography. Water was removed by lyophilization, and distillation of the resultant oil gave 2-D-glycerol. The isopropylidene protecting group was introduced by dissolving 2-D-glycerol in acetone/petroleum ether, adding catalytic amounts of *p*-toluenesulfonic acid, and refluxing the solution with a Dean–Stark trap (Renoll & Newman, 1955). Product **4** was purified by high-vacuum distillation.



4

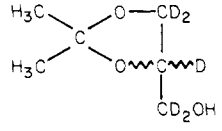
rac-Isopropylidene[1,3-$^2H_4$]glycerol (**5**). Diethyl malonate was oxidized with lead tetraacetate in glacial acetic acid to



5

diethyl acetoxymalonate (Gortatowski & Armstrong, 1957). Reduction with $LiAlD_4$ followed by hydrolysis yielded [1,3-$^2H_4$]glycerol, which was obtained following mixed-bed ion-exchange chromatography, lyophilization, and distillation (Gidez & Karnovsky, 1952). The isopropylidene derivative was prepared as described above.

rac-Isopropylidene[1,2,3-$^2H_5$]glycerol (**6**). Commercially



6

available perdeuterated glycerol (Merck, Sharp and Dohme, Canada) was reacted with acetone as described above. All isopropylideneglycerols were >99% pure as evidenced by gas chromatography. The structures were further verified by their infrared and proton NMR spectra.

Coupling of 2,3-*O*-isopropylideneglycerol with 1,2-dipalmitoylphosphatidic acid and removal of the protecting group yield 1,2-dipalmitoyl-*sn*-glycero-3-phospho-1′-*sn*-glycerol (3,1′-DPPG) which has the naturally occurring L,D configuration. Likewise, 1,2-*O*-isopropylideneglycerol leads to 1,2-dipalmitoyl-*sn*-glycero-3-phospho-3′-*sn*-glycerol (3,3′-DPPG) which has the L,L configuration.

Sample Preparation. Dispersions of DPPG were prepared by mixing the lipid with an excess of buffer, 0.01 M Pipes, pH 7.0, and 0.1 M NaCl. Typically, 30 mg of DPPG in the form of the sodium salt was dispersed in 5 mL of buffer, and the mixture was vigorously vortexed at a temperature above the phase transition. The dispersion was then centrifuged at 39100*g* for 30 min (Sorvall SS-34 rotor, 18 000 rpm) to form a pellet. Only the pellet was used for the NMR measurements. The water content of the pellet was about 85 wt %.

In a number of experiments, DPPG sodium salt was simply dispersed in a small amount of 0.1 M NaCl to form a lipid dispersion containing a well-defined amount of lipid (48.5 wt %). According to Tocanne et al. (1974) (Sacrē & Tocanne, 1977), 0.1 M NaCl yields maximum dissociation of DPPG sodium salt. Small differences in the quadrupole splittings of the buffered and the simple 0.1 M NaCl DPPG dispersions have been observed, but the maximum deviation was less then 10%. The nonbuffered dispersion showed a stronger temperature dependence. If not otherwise noted, all results presented in the following will refer to the buffered DPPG dispersion.

Sample Characterization. In the early stages of this work, the phosphorus-31 NMR and spin-label electron paramagnetic resonance spectra showed a second component in addition to the bilayer pattern. The contribution of this apparently micellar phase to the total spectrum increased with temperature. We attribute the occurrence of this nonlamellar phase to minor impurities in the early DPPG preparations. All results reported in the following refer to samples exhibiting pure bilayer characteristics over the whole temperature range investigated, i.e., usually 20–60 °C.

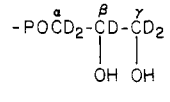
The lamellar structure of the DPPG dispersions was verified by X-ray diffraction. For a powder-type sample of 3,3′-DPPG sodium salt in 0.1 M NaCl (48.5 wt % lipid), the small-angle X-ray diffraction pattern at 47 °C consists of three concentric rings with a ratio of the diameters of 1:2:3. The repeat distance of this lamellar L_α phase is determined to be 49.5 Å. For **3**-*rac*-DPPG in pure water (73 wt % lipid), a repeat distance of d = 57.1 Å at 49 °C has been reported (Ranck et al., 1977). Differential scanning calorimetry of 3,3′-DPPG sodium salt in 0.1 M NaCl solution (48.5 wt % lipid) by using a Perkin-Elmer DSC-2 calorimeter (heating rate 1.25 °C/min) showed a very small endothermic pretransition at 33.9 °C and a major endothermic phase transition at 41.6 °C. The transition enthalpy of this gel to liquid-crystal phase transition was 8.95 kcal/mol of lipid, in agreement with earlier studies on racemic DPPG (e.g., Findlay & Barton, 1978).

NMR Measurements. The phosphorus-31 NMR spectra (36.4 MHz) were recorded on a Bruker HX-90-FT instrument with a home-built quadrature detection unit. The pulse width was about 4 μs, the recycle time 210 ms, and the spectral width 10 kHz. Proton decoupling was achieved by applying 5 W of proton-decoupling power. Deuterium NMR spectra were obtained on a Bruker WH-400-FT instrument (deuterium frequency 61.4 MHz) operated in the quadrature detection mode. The pulse width for a 90° pulse was 19 μs, and the recycle time was 250 ms.

Results

Stereochemical Configuration. In Table I we have compared the specific optical rotation, $[\alpha]^T_\lambda$, of various DPPGs synthesized in this work with some earlier data on synthetic phosphatidylglycerols with saturated fatty acyl chains. Inspection of Table I leads to the conclusion that the stereochemical configuration of the glycerol head group seems to exert a relatively small effect on the optical rotatory power. The specific optical rotation is therefore unsuited to elucidate reliably the stereochemical configuration of the phosphatidylglycerol head group. Instead, we have employed an enzymatic assay to verify the stereochemical purity of the DPPGs synthesized in this work (Haverkate & van Deenen, 1964). DPPG was degraded with phospholiphase C to yield 1,2-dipalmitoyl-*sn*-glycerol and, depending on the DPPG configuration, either *sn*-glycero-1-phosphate (from 3,1′-DPPG) or *sn*-glycero-3-phosphate (from 3,3′-DPPG). Only the latter compound is a substrate for *sn*-glycero-3-phosphate dehydrogenase (Hohorst, 1963), and Figure 1 shows the initial reaction rates of *sn*-glycero-3-phosphate dehydrogenase as a function of total glycerophosphate concentration. The slopes of the three straight lines have the ratios 2:1:0 for 3,3′-DPPG, 3-*rac*-DPPG, and 3,1′-DPPG, respectively, providing conclusive evidence for the indicated stereochemical configurations.

Nuclear Magnetic Resonance Spectra. In the following, the nomenclature α, β, and γ is employed for the three glycerol head-group segments to facilitate comparison with earlier studies on phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine.



The upper part of Figure 2 shows a deuterium NMR spectrum of α-CD_2-3,3′-DPPG randomly dispersed in buffer. The spectrum consists of two overlapping powder patterns of practically equal intensity but slightly different quadrupole splittings. The origin of the two quadrupole splittings could be due to two different head-group conformations or to the motional inequivalence of the two deuterons at the α site. A decision between the two alternatives can be made by comparing α-CD_2-3,3′-DPPG with α-(*R*)-CDH-3,3′-DPPG, where the single deuteron is incorporated stereospecifically by using

Table I: Specific Optical Rotation ($[\alpha]^{T}_{\lambda}$) of Synthetic Phosphatidylglycerols (Sodium Salt)

compd	[α] (deg)	λ (nm)	temp (°C)	solvent	ref
1,2-distearoyl-*sn*-glycero-3-phospho-1′-*sn*-glycerol	9.2	589	25	pyridine	*a*
1,2-dilauroyl-*sn*-glycero-3-phospho-1′-*sn*-glycerol	8.4	589	20	$CHCl_3$/MeOH (1:1 v/v)	*b*
1,2-dipalmitoyl-*sn*-glycero-3-phospho-1′-*sn*-glycerol	7.6	546	30	$CHCl_3$/MeOH (3:1 v/v)	*d*
1,2-dipalmitoyl-*sn*-glycero-3-phospho-3′-*sn*-glycerol	8.6	546	30	$CHCl_3$/MeOH (3:1 v/v)	*d*
1,2-dipalmitoyl-*sn*-glycero-3-phospho-*rac*-glycerol	8.4	546	30	$CHCl_3$/MeOH (3:1 v/v)	*d*
1,2-dipalmitoyl-*sn*-glycero-3-phospho-*rac*-glycerol	6.3	589	21	$CHCl_3$/MeOH (2:1 v/v)	*c*

[a] Saunders & Schwarz (1966). [b] Tocanne et al. (1974c). [c] Watts et al. (1978). [d] This work.

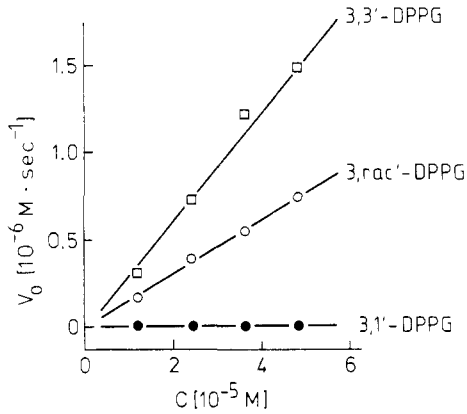


FIGURE 1: From each of the above phospholipids, 2.1 mg was hydrolyzed with phospholipase C according to Haverkate & van Deenen (1964). The diglyceride was extracted with diethyl ether, and the concentration of *sn*-glycero-3-phosphate in the aqueous phases of the hydrolysates of 3,3′-DPPG, 3-*rac*-DPPG, and 3,1′-DPPG was compared by reaction with the enzyme *sn*-glycero-3-phosphate dehydrogenase. Optimal conditions for these measurements were determined with commercial *sn*-glycero-3-phosphate. A plot of the initial reaction rate (V_0) vs. total glycerophosphate concentration (C) yields three straight lines, the slopes of which are in the ratio 2:1:0 for the hydrolysates of 3,3′-DPPG/3-*rac*-DPPG/3,1′-DPPG.

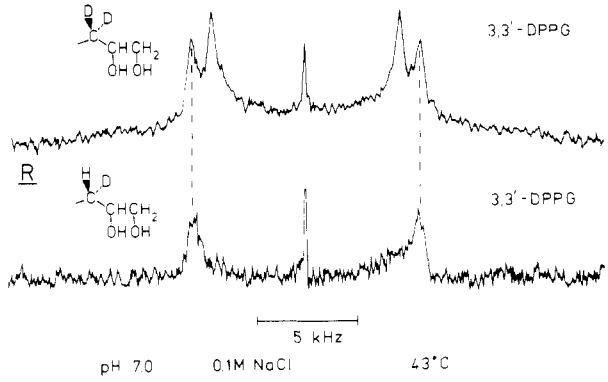


FIGURE 2: Deuterium magnetic resonance spectra (61.4 MHz) of unsonicated 3,3′-DPPG bilayers (15 wt %) in excess Pipes buffer (pH 7.0) and 0.1 M NaCl. Each sample contains 30 mg of phospholipid (dry wt), temperature 43 °C. Upper spectrum: α-dideuterated 3,3′-DPPG; pulse width, 16 μs; number of scans, 2500; spectral width, 50 kHz. Lower spectrum: monolabeled 3,3′-DPPG. The deuteron at the α site was introduced stereospecifically by an enzymatic method as described in text. Spectrometer settings: pulse width, 16 μs; number of scans, 3000; spectral width, 50 kHz.

the enzymatic procedure described above. As is demonstrated in Figure 2 (lower spectrum), only one quadrupole splitting is observed in the monodeuterated compound, and it can be concluded that the two quadrupole splittings of α-CD_2-3,3′-DPPG are indeed caused by some small motional inequivalence of the two deuterons. Interestingly, the difference between the two deuterons at the α site is no longer detectable for the diastereomeric compound 3,1′-DPPG. As is shown in Figure 3, the deuterium NMR spectrum of α-CD_2-3,1′-DPPG is

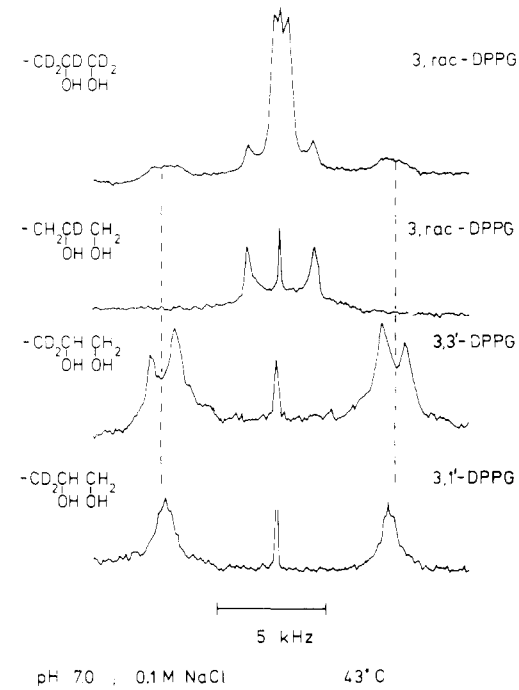


FIGURE 3: Deuterium magnetic resonance spectra (61.4 MHz) of unsonicated DPPG bilayers (15 wt %) in excess Pipes buffer (pH 7.0) and 100 mM NaCl at 43 °C. Each sample contained 30 mg of phospholipid (dry weight).

characterized by just one quadrupole splitting, the size of which corresponds to the average value of the two quadrupole splittings of α-CD_2-3,3′-DPPG. β-CD-3-*rac*-DPPG (which is a mixture of β-CD-3,3′- and β-CD-3,1′-DPPG) is also characterized by just one quadrupole splitting, indicating that at the asymmetric carbon atom the two diastereomers behave motionally identically. From the spectra of Figure 3, it follows by exclusion that the smallest splitting observed in perdeuterated DPPG (uppermost spectrum in Figure 3) must be assigned to the γ-CD_2 group. This is confirmed by the results obtained for α-CD_2-, γ-CD_2-3-*rac*-DPPG (spectra not shown). The combination of the various deuterated DPPGs allows an unambiguous assignment of the observed quadrupole splittings to the three head-group segments, and Figure 4 summarizes the presently available results. In the figure, the quadrupole splittings of the glycerol head-group segments are plotted as a function of temperature. The gel to liquid-crystal phase transition at T_c = 41 °C (cf. Findlay & Barton, 1978) is indicated by a distinct broadening of the deuterium NMR signal below this temperature. It should be noted that the γ position in α,γ deuterated 3-*rac*-DPPG also gives rise to two quadrupole splittings, the possible origin of which will be discussed below.

Finally, we have investigated the motional behavior of the phosphate group by measuring the phosphorus-31 chemical shielding anisotropy. The spectra are characterized by typical

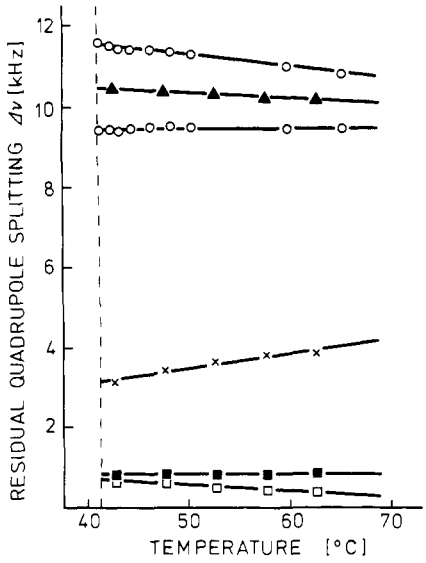


FIGURE 4: Variation of the residual quadrupole splittings of deuterated DPPG bilayers with temperature. DPPG (15 wt %) in excess Pipes buffer (pH 7.0) and 100 mM NaCl. Deuterium labeling of the glycerol segments: (○) α deuterons in 3,3′-DPPG, (▲) α deuterons in 3,1′-DPPG, (×) β deuterons in 3-*rac*-DPPG, (■ and □) γ deuterons in 3-*rac*-DPPG.

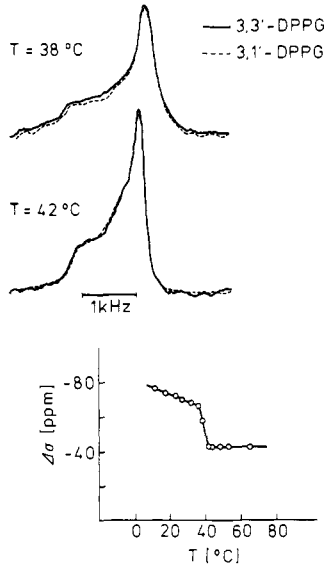


FIGURE 5: Proton-decoupled phosphorus-31 NMR spectra at 36.4 MHz of unsonicated bilayers of the diastereomers 3,3′- and 3,1′-DPPG above and below the phase transition temperature of 41.5 °C.

bilayer patterns (Seelig, 1978), as is illustrated in Figure 5. Within experimental error, the two diastereomers, 3,3′-DPPG (solid line) and 3,1′-DPPG (dashed line), exhibit identical phosphorus-31 NMR spectra over the whole temperature range investigated. The chemical shielding anisotropy is relatively constant above T_c ($\Delta\sigma \simeq -41$ ppm) but increases dramatically below T_c.

Discussion

Motional Inequivalence of Geminal Deuterons. The spectrum of perdeuterated DPPG clearly demonstrates the power of deuterium NMR for the investigation of the head-group region. Not only is it possible to resolve and assign the three head-group CD_2 segments, but even the individual deuterons of the same CD_2 segment can be detected under certain circumstances. The observation that a CD_2 group gives rise to two quadrupole splittings is the exception rather than the rule; most CD_2 groups of phospholipids are characterized by just one quadrupole splitting (cf. Seelig, 1977). Two quadrupole splittings have been encountered so far for only three types of CD_2 groups: (1) the C-2′ segment of fatty acyl chains attached at the phospholipid *sn*-2 position (Seelig & Seelig, 1975; Seelig & Browning, 1978; Gally et al., 1979; Rance et al., 1980), (2) the *sn*-3 segment of the phospholipid–glycerol backbone (Gally et al., 1975; Browning & Seelig, 1980; R. G. Griffin, unpublished experiments), and (3) the α-CD_2 head-group segment of phosphatidylcholine (Gally et al., 1975; Brown & Seelig, 1978), phosphatidylethanolamine (H. Akutsu and J. Seelig, unpublished experiments), phosphatidylglycerol (this work), and phosphatidylserine (Browning & Seelig, 1980). For the α site of 3,3′-DPPG, the stereospecific introduction of just one deuteron results in only one quadrupole splitting. This is the first unambiguous proof that the two quadrupole splittings of a double-deuterated polar head-group α site must be assigned to the two deuterons and are not the result of a slow conformational equilibrium between the two head-group structures. It is likely that the same explanation also applies to all other cases mentioned above, although only the synthesis of stereospecifically monodeuterated compounds will unambiguously confirm this hypothesis.

Head-Group Similarity of 3,3′- and 3,1′-DPPG. The stereochemical configuration of the glycerol head group has relatively little effect on the head-group motion in the lipid bilayer as revealed by the spectral characteristics of the head-group segments. The phosphorus-31 NMR spectra of 3,3′-DPPG and 3,1′-DPPG are virtually identical, as are the deuterium quadrupole splittings at the β position. The most obvious difference is noted for the α position where 3,3′-DPPG exhibits two quadrupole splittings of $\sim$11.5 and 9.5 kHz at 45 °C whereas the 3,1′ diastereomer gives rise to one splitting of 10.5 kHz. However, a difference of $\pm$1 kHz corresponds to a minor change in the spatial anisotropy and should not be interpreted as a distinct structural difference between the two stereoisomers. The spectra of the α site suggest either that the naturally occurring 3,1′ configuration is slightly more flexible than the 3,3′ configuration, so that the small difference of 2 kHz between the two α deuterons in 3,3′-DPPG is motionally averaged in 3,1′-DPPG, or that a small structural change occurs in the torsion angles.

The close similarity of 3,3′-DPPG and 3,1′-DPPG is further indicated by the fact that the stereochemical configuration of the head group has no influence on the gel-to-liquid-crystal transition temperature. Two quadrupole splittings are also observed for the γ position of 3-*rac*-DPPG (Figure 4). Three explanations are possible here. (1) The two deuterons of each γ segment are motionally equivalent, but differences exist between the two diastereomers, 3,3′- and 3,1′-DPPG. (2) The two deuterons are motionally inequivalent, but this inequivalence is identical for 3,3′- and 3,1′-DPPG. (3) A slow conformational equilibrium exists between the two head-group states.

The first possibility can, however, be excluded in all likelihood since ^2H NMR experiments with 3,1′-phosphatidyl[γ-$^2H_2$]glycerol derived from *E. coli* (H. U. Gally, G. Pluschke, P. Overath, and J. Seelig, unpublished experiments) exhibit also two signals of corresponding size and intensity. No decision can be made at present between the remaining alternatives.

The results obtained for DPPG are qualitatively similar to those of 1,2-dimyristoyl-*sn*-glycero-3-phospho-DL-serine (DMPS) (Browning & Seelig, 1980). Small structural differences between the two diastereomers LL- and LD-DMPS have been observed for the α-CD_2 segment whereas identical

Table II: Comparison of the Chemical Shielding Anisotropy, $\Delta\sigma$, and the Deuterium Quadrupole Splittings, $\Delta\nu$, of Phospholipid Head Groups[a]

head group (compd)	T_c (°C)	$\Delta\sigma$ (ppm)	$\lvert\Delta\nu\alpha\rvert$ (kHz)	$\lvert\Delta\nu\beta\rvert$ (kHz)	$\lvert\Delta\nu\gamma\rvert$ (kHz)	ref
phosphocholine[b] (DPPC)	41	−47	6.0	5.3	1.2	*f, g*
phosphoglycerol[c] (3,1′-DPPG)	41	−41.5	10.3	3.3	0.5 0.8	*j*
phosphoethanolamine[d] (DPPE)	63	−41	10.3 9.8	3.7		*h*
phosphoserine[e] (L,L-DMPS)	36	−55	13.7 4.1	15.4		*i*

[a] Data are compared at corresponding temperatures, i.e., 5 °C above the respective gel to liquid-crystal transition temperatures. [b] $POCH_2CH_2N^+(CH_3)_3$. [c] $POCH_2CH(OH)CH_2OH$. [d] $POCH_2CH_2NH_3^+$. [e] $POCH_2CH(COO^-)NH_3^+$. [f] Gally et al. (1975). [g] Seelig et al. (1977). [h] Seelig & Gally (1976). [i] Browning & Seelig (1980). [j] This work.

quadrupole splittings were measured for the β-CD segment. This is surprising since it is the β segment which constitutes the center of asymmetry, and yet the differences are more pronounced at the neighboring α segment.

Motional Properties of the Glycerol Head Group. Table II summarizes the relevant spectroscopic parameters of the four different phospholipid head groups investigated to date. To a first approximation, the head-group motion of phosphatidylglycerol appears to be rather similar to that of phosphatidylcholine and phosphatidylethanolamine despite the fact that phosphatidylglycerol formally carries a net negative charge whereas phosphatidylcholine and phosphatidylethanolamine are zwitterionic. In all three lipids, the chemical shielding anisotropy, $\Delta\sigma$, ranges between −41 and −45 ppm, and the quadrupole splittings decrease gradually with increasing separation between the phosphate group and the CD_2 segment. By contrast, the $\Delta\sigma$ parameter in phosphatidylserine is distinctly larger ($\Delta\sigma$ = −55 ppm), and the quadrupole splittings increase toward the NH_3 terminal of the serine moiety, suggesting a rather rigid head group for phosphatidylserine (Browning & Seelig, 1980).

The quantitative interpretation of the $\Delta\sigma$ and $\Delta\nu$ parameters in terms of a structural model is difficult. At first sight, it could be argued that a decrease in the quadrupole splittings corresponds to an increase in the motional freedom toward the terminal head-group segments. On the other hand, we have shown for phosphatidylcholine and phosphatidylethanolamine that the spectroscopic head-group parameters can be accounted for quantitatively by a relatively rigid head group. The model only assumes relatively fast jumps of the phosphocholine and phosphoethanolamine residues between two mirror-like states such that all torsion angles change their sign. In terms of this model, the torsion angles of the various head-group linkages are found to be consistent with or close to the torsion angles determined by X-ray crystallographic analysis of model compounds (cf. Seelig & Gally, 1976; Seelig et al., 1977; Pearson & Pascher, 1979). No similar analysis is attempted here for the glycerol head group since the only available X-ray analysis is that of a fairly distant model compound, i.e., a racemic mixture of glycerophosphate (Fenn & Marshall, 1972; Taga et al., 1972). However, from the temperature dependence of the DPPG quadrupole splittings, it can be concluded that a purely motional model is not sufficient but that geometric effects must also be invoked. It may be noted from Figure 4 that the quadrupole splitting of the β segment *increases* with temperature. Such a behavior is unusual since an increase in temperature is expected to lead to a more random movement and thus to smaller quadrupole splittings. However, in terms of a geometrical model, an increase in the quadrupole splitting can easily be explained by a small change in the torsion angles such that the average orientation of the C–D bond vector moves away from the magic angle. The central problem in the analysis of the head-group data is therefore the separation of motional and structural effects. Deuterium relaxation time (T_1) measurements should be particularly fruitful in this respect. While the residual deuterium quadrupole splitting, $\Delta\nu_Q$, is determined by the spatial anisotropy of motion, nuclear relaxation processes provide information on the time scale of the motion. Deuterium relaxation rates, $R_1 = 1/T_1$, are dominated essentially by the rate of reorientation of the segment involved and depend only a little on the ordering (Brown et al., 1979). Measurement of the deuterium T_1 relaxation times should therefore shed some light on the motional relationship of the various head-group segments. Finally, one might also resort to high-resolution spectra of phosphatidylglycerol in organic solvents (Marsh & Watts, 1978; cf. also Hosur & Govil, 1979). For a critical evaluation of the spectral analysis presented in the literature, it should be noted that a very specific head-group model is postulated a priori (trans–gauche isomerization) and that the experimental results are used to determine the free parameters of this model. Nevertheless, the high-resolution data at least seem to indicate that the glycerol moiety in solution has a dynamic structure involving more than one conformer.

Earlier investigations have emphasized the effect of surface charge on the molecular properties of lipid bilayers even though no detailed treatment was attempted. The dissimilarity in the head-group parameters of the negatively charged phospholipid head groups phosphoserine and phosphoglycerol on the one hand and the similarity between phosphoglycerol, phosphocholine, and phosphoethanolamine on the other hand suggest, however, that the net negative charge in phosphatidylglycerol is of less importance than anticipated earlier. Much more attention should probably be given to hydrogen bonding and head-group hydration forces before a quantitative understanding of phospholipid head-group properties can be achieved.

References

Aneja, R., Chadha, J. S., & Davies, A. P. (1970) *Biochim. Biophys. Acta 218*, 102–111.

Baer, E. (1951) *J. Biol. Chem. 189*, 235–247.

Baer, E. (1952) *Biochem. Prep. 2*, 31–38.

Baer, E., & Fischer, H. O. L. (1939a) *J. Am. Chem. Soc. 61*, 761–765.

Baer, E., & Fischer, H. O. L. (1939b) *J. Biol. Chem. 128*, 463–473.

Baer, E., & Buchnea, D. (1958) *J. Biol. Chem. 232*, 895–901.

Bentley, R. (1970) *Molecular Asymmetry in Biology*, Vol. II, p 1, Academic Press, New York.

Bonsen, P. P. M., de Haas, G. H., & van Deenen, L. L. M. (1966) *Chem. Phys. Lipids 1*, 33–40.

Brown, M. F., & Seelig, J. (1978) *Biochemistry 17*, 381–384.

Brown, M. F., Seelig, J., & Häberlen, U. (1979) *J. Chem. Phys. 70*, 5045–5053.

Browning, J., & Seelig, J. (1979) *Chem. Phys. Lipids 24*, 103–118.

Browning, J., & Seelig, J. (1980) *Biochemistry 19*, 1262–1270.

El Mashak, E. M., & Tocanne, J. F. (1979) *J. Colloid Interface Sci. 70*, 56–66.

Fenn, R. H., & Marshall, G. E. (1972) *Biochem. J. 130*, 1–10.

Findlay, E. J., & Barton, P. G. (1978) *Biochemistry 17*, 2400–2405.

Gally, H. U., Niederberger, W., & Seelig, J. (1975) *Biochemistry 14*, 3647–3652.
Gally, H. U., Overath, P., Pluschke, G., & Seelig, J. (1979) *Biochemistry 18*, 5605–5610.
Gidez, L. I., & Karnovsky, M. I. (1952) *J. Am. Chem. Soc. 74*, 2413–2415.
Gortatowski, M. J., & Armstrong, M. D. (1957) *J. Org. Chem. 22*, 1217–1220.
Günther, H., Biller, F., Kellner, M., & Simon, H. (1973) *Angew. Chem. 85*, 141–142.
Haverkate, F., & van Deenen, L. L. M. (1964) *Biochim. Biophys. Acta 84*, 106–108.
Hohorst, H. J. (1963) *Methods of Enzymatic Analysis*, p 215.
Hosur, R. V., & Govil, G. (1979) *Curr. Sci. 48*, 329–332.
Jacobson, K., & Papahadjopoulos, D. (1975) *Biochemistry 14*, 152–161.
Joutti, A., & Renkonen, O. (1976) *Chem. Phys. Lipids 17*, 264–266.
Kiyasu, J. Y., Pieringer, R. A., Paulus, H., & Kennedy, E. P. (1963) *J. Biol. Chem. 238*, 2293–2298.
Lammers, J. G., & van Boom, J. H. (1977) *Recl. Trav. Chim. Pays-Bas 96*, 216–218.
Lammers, J. G., & van Boom, J. H. (1979) *Recl. Trav. Chim. Pays-Bas 98*, 243–250.
Lecocq, J., & Ballou, C. E. (1964) *Biochemistry 3*, 976–980.
Lok, C. M., Ward, J. P., & van Dorp, D. A. (1976) *Chem. Phys. Lipids 16*, 115–122.
Marsh, D., & Watts, A. (1978) *FEBS Lett. 85*, 124–126.
Mattson, F. H., & Volpenheim, R. A. (1962) *J. Lipid Res. 3*, 281–296.
Papahadjopoulos, D. (1977) *J. Colloid Interface Sci. 58*, 459–470.
Papahadjopoulos, D., Jacobson, K., Nir, S., & Isac, T. (1973) *Biochim. Biophys. Acta 311*, 330–348.
Papahadjopoulos, D., Moscarello, M., Eylar, E. H., & Isac, T. (1975) *Biochim. Biophys. Acta 401*, 317–335.
Pearson, R. H., & Pasher, I. (1979) *Nature (London) 281*, 499–501.
Rance, M., Jeffrey, K. R., Tulloch, A. P., Butler, K. W., & Smith, I. C. P. (1980) *Biochim. Biophys. Acta*, in press.
Ranck, J. L., Keira, T., & Luzzati, V. (1977) *Biochim. Biophys. Acta 488*, 432–441.
Reichstein, T., Pedolin, A., & Grüssner, A. (1935) *Helv. Chim. Acta 18*, 598.
Renoll, M., & Newman, M. S. (1955) *Organic Syntheses*, Collect. Vol. 3, pp 502–504, Wiley, New York.
Rottem, S., & Markowitz, O. (1979) *Biochemistry 18*, 2930–2935.
Sacrē, M. M., & Tocanne, J. F. (1977) *Chem. Phys. Lipids 18*, 334–354.
Saunders, R. M., & Schwarz, H. P. (1966), *J. Am. Chem. Soc. 88*, 3844–3847.
Seelig, J. (1977) *Q. Rev. Biophys. 10*, 353–418.
Seelig, J. (1978) *Biochim. Biophys. Acta 505*, 105–141.
Seelig, A., & Seelig, J. (1975) *Biochim. Biophys. Acta 406*, 1–5.
Seelig, J., & Gally, H. (1976) *Biochemistry 15*, 5199–5204.
Seelig, J., & Browning, J. L. (1978) *FEBS Lett. 92*, 41–44.
Seelig, J., Gally, H., & Wohlgemuth, R. (1977) *Biochim. Biophys. Acta 467*, 109–119.
Taga, T., Senma, M., & Osaki, K. (1972) *J. Chem. Soc., Chem. Commun.*, 465–466.
Tocanne, J. F., Ververgaert, P. H. J. Th., Verkleij, A. J., & van Deenen, L. L. M. (1974a) *Chem. Phys. Lipids 12*, 201–219.
Tocanne, J. F., Ververgaert, P. H. J. Th., Verkleij, A. J., & van Deenen, L. L. M. (1974b) *Chem. Phys. Lipids 12*, 220–231.
Tocanne, J. F., Verheij, H. M., op den Kamp, J. A. F., & van Deenen, L. L. M. (1974c) *Chem. Phys. Lipids 13*, 389–403.
van Dijck, P. W. M., Ververgaert, P. H. J. Th., Verkleij, A. J., van Deenen, L. L. M., & de Gier, J. (1975) *Biochim. Biophys. Acta 406*, 465–478.
van Dijck, P. W. M., De Kruijff, B., Verkleij, A. J., van Deenen, L. L. M., & de Gier, J. (1978) *Biochim. Biophys. Acta 512*, 84–96.
Verkleij, A. J., de Kruijff, B., Ververgaert, P. H. J. Th., Tocanne, J. F., & van Deenen, L. L. M. (1974) *Biochim. Biophys. Acta 339*, 432–437.
Ververgaert, P. H. J. Th., de Kruijff, B., Verkleij, A. J., Tocanne, J. F., & van Deenen, L. L. M. (1975) *Chem. Phys. Lipids 14*, 97–101.
Watts, A., Harlos, K., Maschke, W., & Marsh, D. (1978) *Biochim. Biophys. Acta 510*, 63–74.