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# Conformation and packing properties of membrane lipids: The crystal structure of sodium dimyristoylphosphatidylglycerol

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**The conformation and molecular packing of sodium 1,2-dimyristoyl-*sn*-glycero-phospho-*rac*-glycerol (DMPG) have been determined by single crystal analysis ($R = 0.098$). The lipid crystallizes in the monoclinic spacegroup $P2_1$ with the unit cell dimensions $a = 10.4$, $b = 8.5$, $c = 45.5$ Å and $\beta = 95.2°$. There are two independent molecules (A and B) in the asymmetric unit which with respect to configuration and conformation of their glycerol headgroup are mirror images. The molecules pack tail to tail in a bilayer structure. The phosphoglycerol headgroups have a layer-parallel orientation giving the molecules an L-shape. At the bilayer surface the (−) phosphoglycerol groups are arranged in rows which are separated by rows of (+) sodium ions. Laterally the polar groups interact by an extensive network of hydrogen, ionic and coordination bonds. The packing cross-section per molecule is 44.0 Å$^2$. The hydrocarbon chains are tilted (29°) and have opposite inclination in the two bilayer halves. In the chain matrix the chain planes are arranged according to a so far unknown hybride packing mode which combines the features of $T_{\parallel}$ and $O_{\perp}$ subcells. The two fatty acid substituted glycerol oxygens have mutually a − synclinal rather than the more common + synclinal conformation. The conformation of the diacylglycerol part of molecule A and B is distinguished by an axial displacement of the two hydrocarbon chains by four methylene units. This results in a reorientation of the glycerol back bone and a change in the conformation and stacking of the hydrocarbon chains. In molecule A the β-chain is straight and the γ-chain is bent while in molecule B the chain conformation is reversed.**

## Introduction

Phosphatidylglycerol (PG) is an abundant lipid in the plasmamembrane of microorganisms [1,2] and in the chloroplast membrane of plants [3]. In mammalian systems PG occurs in minor amounts only, notably in lung surfactants [4,5] and in mitochondrial membranes [6], where it can function as a precursor of cardiolipin [7].

Like other negatively-charged phospholipids, PG exhibits a very complex phase behaviour in aqueous media. The phase transitions and structural organization of PG are affected both by temperature, surface pH and concentration of monovalent and divalent cations [8–15]. Depending on the environmental conditions (proton, ion,

Abbreviations: DMPG, dimyristoylphosphatidylglycerol; DPPG, dipalmitoylphosphatidylglycerol; PG, phosphatidylglycerol; PC, phosphatidylcholine; sc, synclinal ( = *gauche*); ac, anticlinal ( = anti-*gauche*); ap, antiperiplanar (*trans*-planar).

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TABLE I

COORDINATES AND EQUIVALENT ISOTROPIC TEMPERATURE FACTORS

Atomic fractional coordinates and equivalent isotropic temperature factors ($\times 10^2$) for the non-hydrogen atoms of DMPG. $U_{eq} = 1/3(U_{11} + U_{22} + U_{33} + 2^* U_{13}^* \cos \beta)$.

| Molecule A | | | | |
|---|---|---|---|---|
| Atom | *X* | *Y* | *Z* | $U_{eq}$ |
| Na (1) | 0.1593 (9) | 0.4944 (14) | 0.0075 (3) | 4.7 (0.7) |
| P (1) | 0.0790 (7) | 0.1754 (10) | 0.0391 (2) | 4.1 (0.5) |
| O (11) | 0.1192 (20) | 0.1510 (25) | 0.0731 (4) | 5.3 (1.3) |
| O (12) | −0.0131 (19) | 0.3275 (23) | 0.0381 (5) | 5.0 (1.3) |
| O (13) | 0.0049 (17) | 0.0226 (23) | 0.0298 (5) | 6.0 (1.4) |
| O (14) | 0.1883 (16) | 0.2313 (25) | 0.0226 (5) | 5.6 (1.3) |
| C (11) | −0.1521 (26) | 0.3090 (39) | 0.0417 (8) | 5.7 (2.2) |
| C (12) | −0.1908 (24) | 0.4541 (41) | 0.0598 (7) | 4.9 (1.9) |
| C (13) | −0.3406 (33) | 0.4364 (40) | 0.0639 (9) | 6.8 (2.3) |
| O (15) | −0.1717 (20) | 0.6001 (25) | 0.0441 (5) | 5.6 (1.4) |
| O (16) | −0.4172 (18) | 0.4488 (28) | 0.0379 (5) | 5.6 (1.3) |
| C (1) | 0.2181 (37) | 0.2613 (46) | 0.0866 (12) | 10.6 (3.3) |
| C (2) | 0.3033 (51) | 0.1729 (64) | 0.1109 (10) | 12.2 (3.8) |
| C (3) | 0.4403 (30) | 0.2402 (44) | 0.1151 (10) | 8.1 (2.5) |
| O (21) | 0.2324 (22) | 0.1610 (35) | 0.1382 (6) | 8.0 (1.8) |
| O (22) | 0.3546 (25) | −0.0469 (40) | 0.1537 (7) | 10.5 (2.3) |
| C (21) | 0.2740 (45) | 0.0474 (59) | 0.1573 (10) | 8.3 (3.2) |
| C (22) | 0.1975 (35) | 0.0559 (44) | 0.1828 (8) | 7.0 (2.4) |
| C (23) | 0.2234 (34) | −0.0644 (57) | 0.2067 (9) | 9.0 (2.8) |
| C (24) | 0.1434 (37) | −0.0569 (65) | 0.2330 (9) | 10.0 (3.2) |
| C (25) | 0.1866 (41) | −0.1868 (63) | 0.2554 (9) | 10.1 (3.3) |
| C (26) | 0.1092 (45) | −0.1843 (69) | 0.2809 (12) | 12.0 (3.9) |
| C (27) | 0.1384 (39) | −0.3100 (65) | 0.3035 (8) | 9.8 (3.2) |
| C (28) | 0.0656 (36) | −0.3090 (63) | 0.3311 (10) | 10.0 (3.2) |
| C (29) | 0.0958 (47) | −0.4339 (62) | 0.3530 (9) | 11.3 (3.6) |
| C (210) | 0.0239 (48) | −0.4350 (69) | 0.3791 (10) | 12.2 (3.9) |
| C (211) | 0.0636 (49) | −0.5389 (89) | 0.4032 (10) | 14.1 (4.5) |
| C (212) | −0.0031 (68) | −0.5506 (105) | 0.4301 (15) | 18.7 (6.4) |
| C (213) | 0.0224 (67) | −0.6426 (131) | 0.4583 (18) | 22.0 (8.1) |
| C (214) | −0.0490 (79) | −0.6283 (118) | 0.4843 (15) | 22.0 (8.4) |
| O (31) | 0.4140 (25) | 0.4033 (32) | 0.1258 (6) | 9.4 (1.9) |
| O (32) | 0.6302 (27) | 0.4079 (48) | 0.1391 (10) | 13.7 (2.9) |
| C (31) | 0.5301 (64) | 0.4729 (66) | 0.1389 (10) | 11.9 (4.2) |
| C (32) | 0.4805 (48) | 0.6121 (52) | 0.1520 (12) | 10.6 (3.6) |
| C (33) | 0.4899 (55) | 0.6050 (55) | 0.1852 (13) | 12.4 (4.2) |
| C (34) | 0.4372 (38) | 0.4922 (54) | 0.2000 (9) | 7.7 (2.7) |
| C (35) | 0.4441 (48) | 0.4752 (65) | 0.2308 (13) | 12.2 (4.1) |
| C (36) | 0.3899 (47) | 0.3542 (61) | 0.2480 (10) | 11.1 (3.6) |
| C (37) | 0.4051 (45) | 0.3496 (69) | 0.2802 (11) | 11.4 (3.9) |
| C (38) | 0.3510 (43) | 0.2285 (75) | 0.2969 (13) | 11.4 (4.0) |
| C (39) | 0.3643 (58) | 0.2136 (70) | 0.3279 (16) | 14.5 (5.0) |
| C (310) | 0.3080 (44) | 0.1049 (60) | 0.3442 (13) | 10.3 (3.6) |
| C (311) | 0.3289 (59) | 0.0881 (95) | 0.3768 (15) | 16.2 (5.9) |
| C (312) | 0.2683 (66) | −0.0047 (95) | 0.3954 (14) | 16.1 (5.8) |
| C (313) | 0.2900 (63) | −0.0258 (89) | 0.4308 (21) | 18.2 (6.7) |
| C (314) | 0.2200 (90) | −0.1420 (122) | 0.4437 (22) | 24.2 (9.3) |

Molecule B

| Atom | | X | Y | Z | $U_{eq}$ |
|---|---|---|---|---|---|
| Na | (2) | 0.6580 (9) | −0.3216 (14) | 0.0081 (2) | 4.2 (0.6) |
| P | (1) | 0.5778 (7) | 0.0000 (-) | 0.0390 (2) | 4.2 (0.4) |
| O | (11) | 0.6116 (18) | 0.0175 (24) | 0.0730 (4) | 5.1 (1.2) |
| O | (12) | 0.4794 (17) | −0.1549 (24) | 0.0370 (5) | 5.7 (1.4) |
| O | (13) | 0.5097 (16) | 0.1492 (24) | 0.0296 (4) | 4.7 (1.2) |
| O | (14) | 0.6904 (15) | −0.0608 (24) | 0.0239 (4) | 5.2 (1.2) |
| C | (11) | 0.3476 (27) | −0.1235 (37) | 0.0437 (9) | 6.1 (2.2) |
| C | (12) | 0.3104 (27) | −0.2795 (47) | 0.0584 (9) | 7.1 (2.4) |
| C | (13) | 0.1621 (36) | −0.2783 (38) | 0.0641 (9) | 7.0 (2.4) |
| O | (15) | 0.3314 (19) | −0.4190 (25) | 0.0427 (6) | 5.7 (1.4) |
| O | (16) | 0.0839 (19) | −0.2785 (24) | 0.0377 (5) | 4.9 (1.2) |
| C | (1) | 0.6724 (61) | −0.1066 (55) | 0.0918 (9) | 12.1 (3.8) |
| C | (2) | 0.8015 (51) | −0.0500 (82) | 0.1041 (11) | 11.1 (4.1) |
| C | (3) | 0.8018 (44) | 0.0617 (56) | 0.1241 (11) | 9.3 (3.3) |
| O | (21) | 0.8366 (38) | −0.2067 (42) | 0.1228 (9) | 11.6 (2.8) |
| O | (22) | 1.0382 (43) | −0.1420 (63) | 0.1235 (9) | 15.3 (3.9) |
| C | (21) | 0.9583 (89) | −0.2258 (71) | 0.1289 (17) | 12.9 (5.9) |
| C | (22) | 1.0015 (51) | −0.3761 (99) | 0.1472 (13) | 17.1 (5.8) |
| C | (23) | 0.9187 (52) | −0.3694 (59) | 0.1722 (11) | 13.1 (4.2) |
| C | (24) | 0.9712 (41) | −0.5164 (61) | 0.1939 (10) | 10.1 (3.3) |
| C | (25) | 0.9033 (40) | −0.5219 (59) | 0.2205 (13) | 11.4 (3.7) |
| C | (26) | 0.9360 (46) | −0.6473 (61) | 0.2440 (11) | 10.7 (3.6) |
| C | (27) | 0.8649 (46) | −0.6516 (69) | 0.2704 (14) | 13.7 (4.6) |
| C | (28) | 0.9038 (43) | −0.7640 (59) | 0.2939 (10) | 10.3 (3.3) |
| C | (29) | 0.8264 (40) | −0.7625 (59) | 0.3179 (11) | 10.5 (3.3) |
| C | (210) | 0.8629 (50) | −0.8717 (67) | 0.3439 (11) | 11.6 (4.0) |
| C | (211) | 0.7817 (48) | −0.8884 (74) | 0.3667 (13) | 13.4 (4.5) |
| C | (212) | 0.8191 (47) | −0.9873 (69) | 0.3940 (11) | 12.3 (3.9) |
| C | (213) | 0.7429 (63) | −1.0022 (90) | 0.4194 (14) | 17.0 (5.8) |
| C | (214) | 0.7834 (60) | −1.1137 (92) | 0.4421 (13) | 17.7 (5.9) |
| O | (31) | 0.7167 (22) | 0.0450 (31) | 0.1466 (5) | 7.8 (1.7) |
| O | (32) | 0.8714 (33) | 0.0595 (58) | 0.1824 (7) | 15.4 (3.3) |
| C | (31) | 0.7516 (42) | 0.0430 (48) | 0.1733 (10) | 8.9 (3.0) |
| C | (32) | 0.6726 (36) | 0.0292 (56) | 0.1979 (8) | 7.9 (2.7) |
| C | (33) | 0.7179 (31) | −0.0619 (51) | 0.2239 (8) | 6.9 (2.3) |
| C | (34) | 0.6240 (40) | −0.0865 (74) | 0.2476 (9) | 11.1 (3.7) |
| C | (35) | 0.6719 (38) | −0.1818 (61) | 0.2740 (12) | 10.4 (3.5) |
| C | (36) | 0.5914 (41) | −0.2008 (69) | 0.2977 (10) | 10.3 (3.5) |
| C | (37) | 0.6307 (40) | −0.2914 (69) | 0.3227 (11) | 10.8 (3.6) |
| C | (38) | 0.5444 (45) | −0.3108 (85) | 0.3465 (11) | 13.3 (4.3) |
| C | (39) | 0.5922 (37) | −0.4229 (68) | 0.3723 (12) | 11.2 (3.7) |
| C | (310) | 0.5117 (56) | −0.4450 (69) | 0.3965 (11) | 12.7 (4.3) |
| C | (311) | 0.5535 (45) | −0.5510 (78) | 0.4210 (13) | 12.6 (4.3) |
| C | (312) | 0.4786 (59) | −0.5684 (91) | 0.4448 (12) | 15.3 (5.0) |
| C | (313) | 0.5093 (66) | −0.6586 (120) | 0.4742 (17) | 20.0 (7.1) |
| C | (314) | 0.4268 (73) | −0.6595 (173) | 0.4942 (14) | 25.9 (9.6) |

and non-electrolyte concentrations) PG was shown to form lamellar structures with great variations in bilayer thickness [10,12]. This indicates a great flexibility in the lateral interactions and packing requirements of the phosphoglycerol headgroup at the bilayer surface. The hydrocarbon chains accommodate to these different molecular cross-sections by forming either layer-perpendicular, tilted or even interdigitating chain matrices [10, 12–15].

In order to obtain insight in interactions and packing properties of PG we have performed a X-ray single crystal analysis of sodium 1,2-dimyristoyl-*sn*-glycerol-3-phospho-*rac*-glycerol.

## Experimental

Monosodium 1,2-dimyristoyl-*sn*-glycerol-3-phospho-*rac*-glycerol,(2,3-dimyristoyl-D-glycerol-1-phospho-DL-glycerol) DMPG (for numbering conventions and configurational notations in structural studies of lipids see Ref. 21) was obtained from Calbiochem (Ca, U.S.A.).

It is of interest to note that attempts to grow crystals of DMPG with natural L (*sn*-1-*P*) configuration at the phosphoglycerol headgroup were so far unsuccessful, while for the corresponding diasteromeric pair with DL (*rac*) phosphoglycerol headgroups suitable crystals for X-ray analysis could be obtained.

For crystallization the lipid was dissolved (1 mg/ml) in a solvent mixture of carbon tetrachloride/ethanol/water (100 : 10 : 0.3, by vol.). The crystallization was induced by diffusion of diethyl ether vapours into the lipid solution. Thin plate-like crystals appeared after several days.

A crystal with the dimensions 0.35 × 0.35 × 0.02 mm was used for data collection with an Enraf-Nonius CAD4F-11 diffractometer. The angular settings of 25 reflections ($8° < \theta < 23°$) were measured to calculate the lattice parameters. Intensity data were collected by the $\theta/2\theta$ scan method using monochromatized $CuK_\alpha$ radiation. Three intensity control reflections were measured every 2 h. At the end of the data collection the intensities of these reflections had dropped to about 90% of their original values. All reflections were rescaled to account for this decay. A total of 5408 independent reflections ($1° < \theta < 55°$) were recorded and of these 1247 reflections with $I > 3\sigma(I)$ were considered observed. All intensities were corrected for Lorentz and polarization effects but not for absorption or extinction.

*Crystal data*

Molecular formula $C_{34}H_{66}O_{10}NaP$, space group $P2_1$, unit cell $a = 10.370(8)$, $b = 8.482(7)$, $c = 45.52(2)$ Å, $\beta = 95.22(4)°$, $V = 3987$ Å$^3$, $Z = 4$, $M_r = 688.16$, $D_c = 1.146$ g · m$^{-3}$, $\mu(CuK_\alpha) = 11.2$ cm$^{-1}$.

The structure was solved by direct methods using the programs DIRDIF [16] and MITHRIL [17] which provided the non-hydrogen atom positions. Refinement was carried out by the full-matrix least-squares method and anisotropic temperature factors were applied for the non-hydrogen atoms. Hydrogen atoms connected to carbon atoms (except those connected to methyl end groups) were included at expected positions and were assigned a common isotropic temperature factor (5 Å$^2$). The hydrogen atom parameters were not refined. The refinement was terminated with residuals $R = 0.098$ and $R_w = 0.115$. The weighting scheme used in the later part of the refinement was $w = 1/(1 + ((|F_{obs}| - 32)/35)^2)$ [18]. The form factors used were those given by Cromer and Mann [19]. All calculations have been performed on a DEC-system-10 computer using mainly the X-ray 72 program system [20].

## Description of the structure

The coordinates of the non-hydrogen atoms are given in Table I. Observed and calculated structure factors, anisotropic temperature parameters and coordinates of the hydrogen atoms can be obtained from the Department of Structural Chemistry, Göteborg University. The numbering of the atoms and notation of torsion angles is shown in Fig. 1. For configurational and conformational notation used in structural studies of glycerophospholipids see Ref. 21.

*Molecular packing*

The DMPG molecules are arranged in a typical bilayer structure. The packing pattern and the extension of the unit cell is shown in Fig. 2 in views along the two short unit cell axes *a* and *b*.

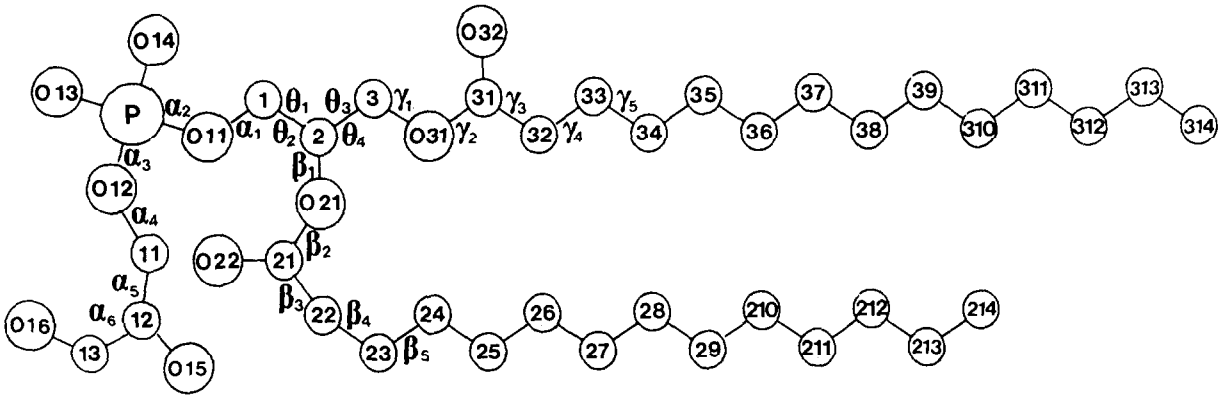


Fig. 1. Schematic drawing of a DMPG molecule showing atom numbering and notation of torsion angles according to the conventions used in conformational studies [21].

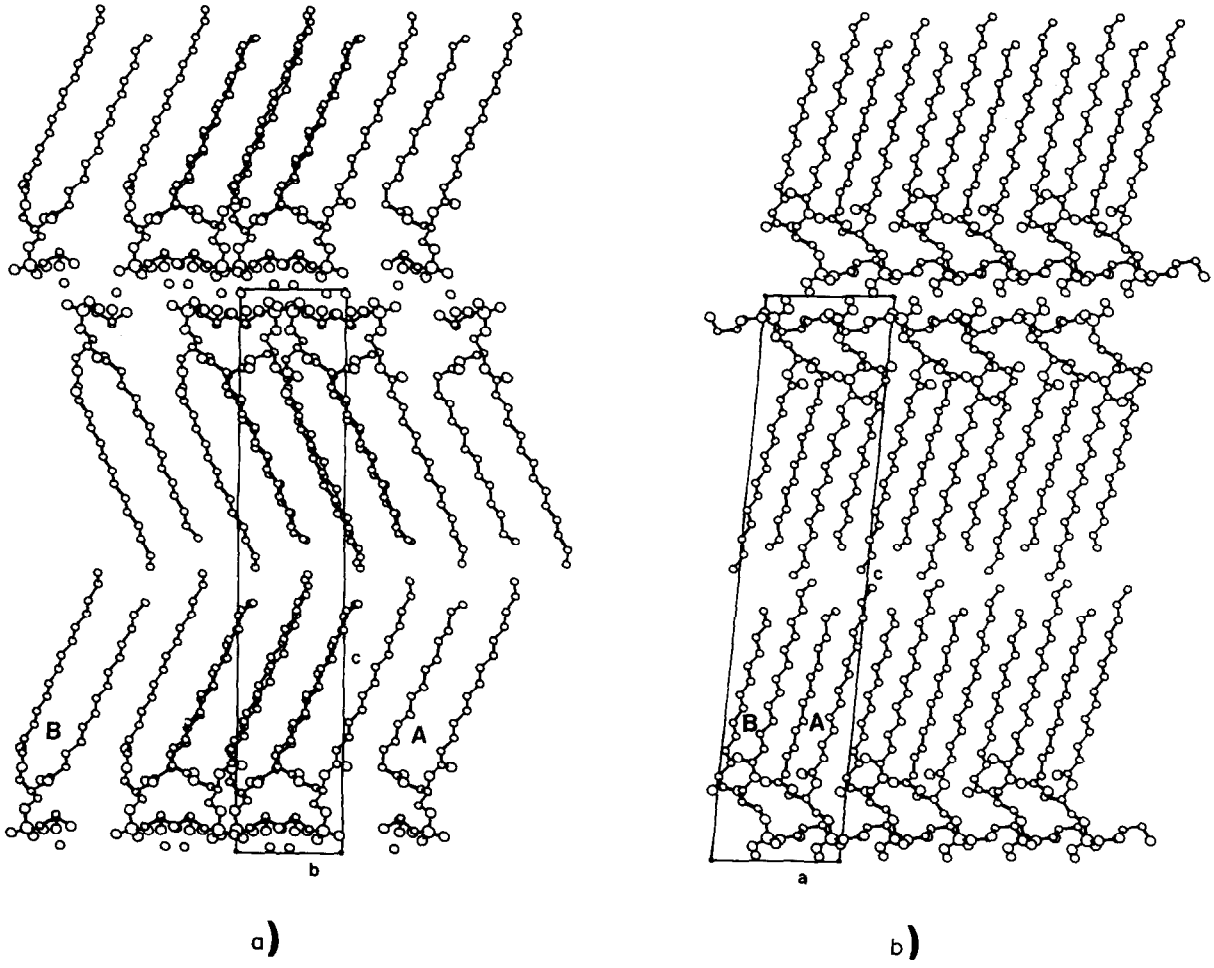


Fig. 2. Molecular packing of DMPG in views (a) along the unit cell *a*-axis and (b) along the *b*-axis. In the projection (a) DMPG molecules A and B overlap (center). For clarity, on the right side of the bilayer only A molecules and on the left side only B molecules are reproduced.

The unit cell contains four molecules, two of which (A and B) constitute the asymmetric unit and are related to the opposite pair by a 2-fold screw axis. The two molecules A and B in each pair are diastereomers, differing with respect to the configuration of their glycerol headgroups. The diacylglycerol moiety has natural D-configuration (*sn*-3-*P*) in both molecules while the glycerol headgroup has natural L-configuration (*sn*-1-*P*) in molecule A, but D-configuration (*sn*-3-*P*) in molecule B. The headgroups of molecule A and B are mirror images also with respect to their conformation (see molecular conformation below).

The phosphoglycerol headgroups are oriented parallel to the bilayer plane forming a rather smooth surface. The headgroup layers of two adjacent bilayers sandwich a layer of sodium ions at the bilayer interface.

The packing requirement of the layer-parallel phosphoglycerol headgroup determines the molecular packing cross-section in the layer plane ($S$ = 44.0 Å$^2$). The hydrocarbon chains accommodate to this headgroup area by adopting a tilt of 29°. The chains thereby are inclined both in direction of the *a*- and *b*-axes. Furthermore, as obvious from Fig. 2a the hydrocarbon chains of the two bilayer halves have opposite tilt direction. In the projection along the *b*-axis (Fig. 2b) the chains appear parallel.

*Chain packing mode*

Laterally the chains pack according to a hitherto unknown hybride packing mode. The chain packing pattern in a view along the chain axes ($c_s$-axis) is shown in Fig. 3. The subcell combines features of both triclinic parallel $T_{\parallel}$ and orthorhombic

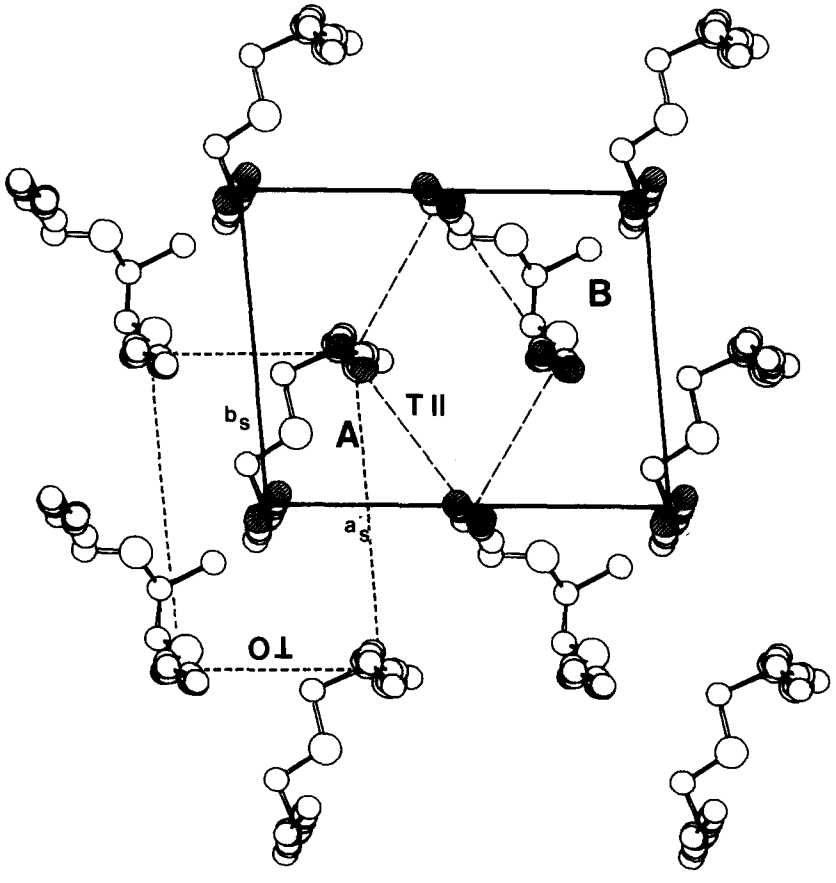


Fig. 3. Lateral packing of the hydrocarbon chains of DMPG in a view along the chain axes ($c_s$-axis). The hybrid subcell (solid lines) combines features of both $T_{\parallel}$ and $O_{\perp}$ chain packing modes (broken lines).

perpendicular $O_\perp$ chain packing modes [22]. The packing pattern can be regarded as a variation of the hybrid subcells (HS1 and HS2) [22] which earlier have been observed in phosphatidylethanolamine [23] and cerebroside [24]. The hybrid subcell of DMPG has the dimensions:
$a_s = 10.1(1)$ Å, $b_s = 7.8(1)$ Å, $c_s = 2.54(3)$ Å and $\alpha_s = 101(2)°$, $\beta_s = 90(1)°$, $\gamma_s = 97(1)°$,
which corresponds to a packing cross-section per chain of $\Sigma = 19.2$ Å$^2$. The parameters of the $T_{||}$ 'sub'-subcell are: $a_s = 4.4(1)$ Å, $b_s = 5.4(1)$ Å, $c_s = 2.54(3)$ Å, $\alpha_s = 67(1)°$, $\beta_s = 105(1)°$, $\gamma_s = 118(2)°$ and of the distorted $O_\perp$ 'sub'-subcell: $a_s = 5.0(1)$ Å, $b_s = 7.8(1)$ Å, $c_s = 2.54(3)$ Å, $\alpha_s = 83(2)°$, $\beta_s = 84(1)°$, $\gamma_s = 92(1)°$.

As obvious from Figs. 3 and 4 the zigzag planes of the two hydrocarbon chains of molecule A are oriented at a right angle to each other, while they are parallel in molecule B. The hybrid subcell thus is constituted of four chains, three with mutually parallel chain planes and one perpendicular to the others.

*Molecular conformation*

The conformation of the two DMPG molecules A and B is shown in Fig. 4 in projections along the unit cell axes *a* and *b*. Torsion angles in comparison to those of other membrane lipids are given in Table II.

*Diacylglycerol part.* The diacylglycerol moiety displays conformational features that recently have been reported for phosphatidyl-*N*,*N*-dimethylethanolamine [25]. Typical for this conformation is that the two fatty acid substituted glycerol oxygens O(21) and O(31) have mutually a −sc ($\theta_4$) rather than the well known +sc position [21,26] (compare $\theta_4$ Table II).

The two DMPG molecules, however, differ with respect to orientation of their glycerol back bone and their chain stacking. In molecule A the C(2)-O(21) glycerol bond points approximately in the direction of the layer normal and the C(3)-O(31) bond has an almost layer-parallel orientation. The adherent β-chain is straightly extended while the γ-chain is bent perpendicularly at carbon atom

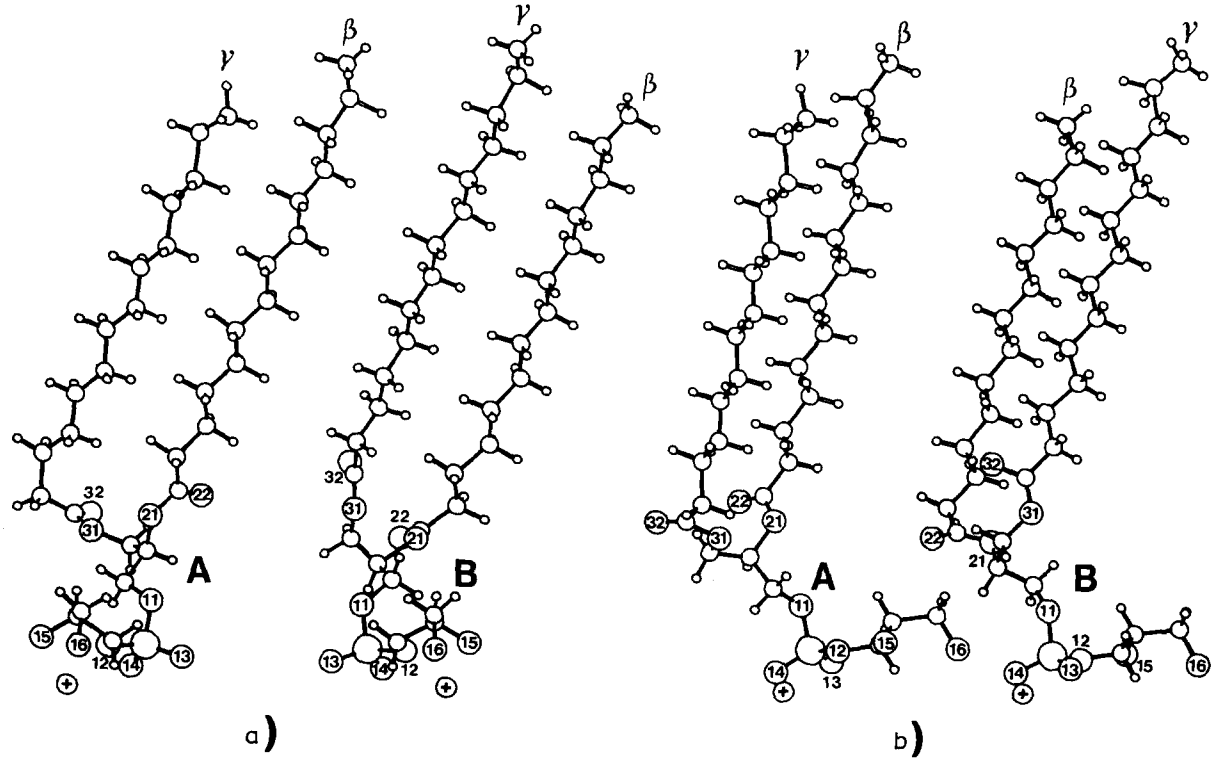


Fig. 4. Molecular conformation of DMPG molecules A and B in views (a) along the unit cell *a*-axis and (b) along the *b*-axis. Note the difference in the orientation of the diacylglycerol part of molecule A and B and the lateral and axial shift of the β and γ chains (projection b).

TABLE II

TORSION ANGLES

For torsion angle notations see Fig. 1. $DLPEM_2$, dilauroylphosphatidyl-*N*,*N*-dimethylethanolamine [25]; DMPC, dimyristoylphosphatidylcholine [27]; DMPA, dimyristoylphosphatidate [28].

| | $\alpha_1$ | $\alpha_2$ | $\alpha_3$ | $\alpha_4$ | $\alpha_5$ | $\alpha_6$ | $\theta_1$ | $\theta_2$ | $\theta_3$ | $\theta_4$ | $\beta_1$ | $\beta_2$ | $\beta_3$ | $\beta_4$ | $\gamma_1$ | $\gamma_2$ | $\gamma_3$ | $\gamma_4$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DMPG A | −146 | −76 | −86 | 143 | 180 | −66 | 151 | −78 | 64 | −63 | 159 | 178 | 178 | −179 | 164 | −170 | 110 | −57 |
| DMPG B | 116 | 58 | 78 | −147 | −173 | 67 | 71 | 179 | 45 | −58 | 157 | 180 | −50 | −175 | 122 | 179 | 142 | 174 |
| $DLPEM_2$ | 179 | 65 | 54 | 144 | −96 | | 176 | −66 | 56 | −60 | 148 | 173 | −57 | 176 | 129 | −167 | 166 | 175 |
| DMPC | 177 | −74 | −47 | −150 | 54 | | 168 | −80 | 166 | 51 | 120 | 179 | −134 | 67 | 102 | 176 | 180 | 180 |
| DMPA | 153 | | | | | | −54 | 62 | −179 | 62 | 87 | 172 | 164 | 179 | −142 | 180 | −119 | 73 |

C(31). This bend is produced by a +ac ($\gamma_3 = 110°$) and −sc ($\gamma_4 = -57°$) twist about the C(31)-C(32) and C(32)-C(33) bond, respectively.

Compared to molecule A the glycerol backbone of molecule B is turned by approximately 60° (see Fig. 5). This changes the orientation of the glycerol bonds C(2)-O(21) and C(3)-O(31) form 'vertical' to 'horizontal' and vice versa. Thereby the two hydrocarbon chains get axially displaced by four methylene units. The β-chain becomes bent and

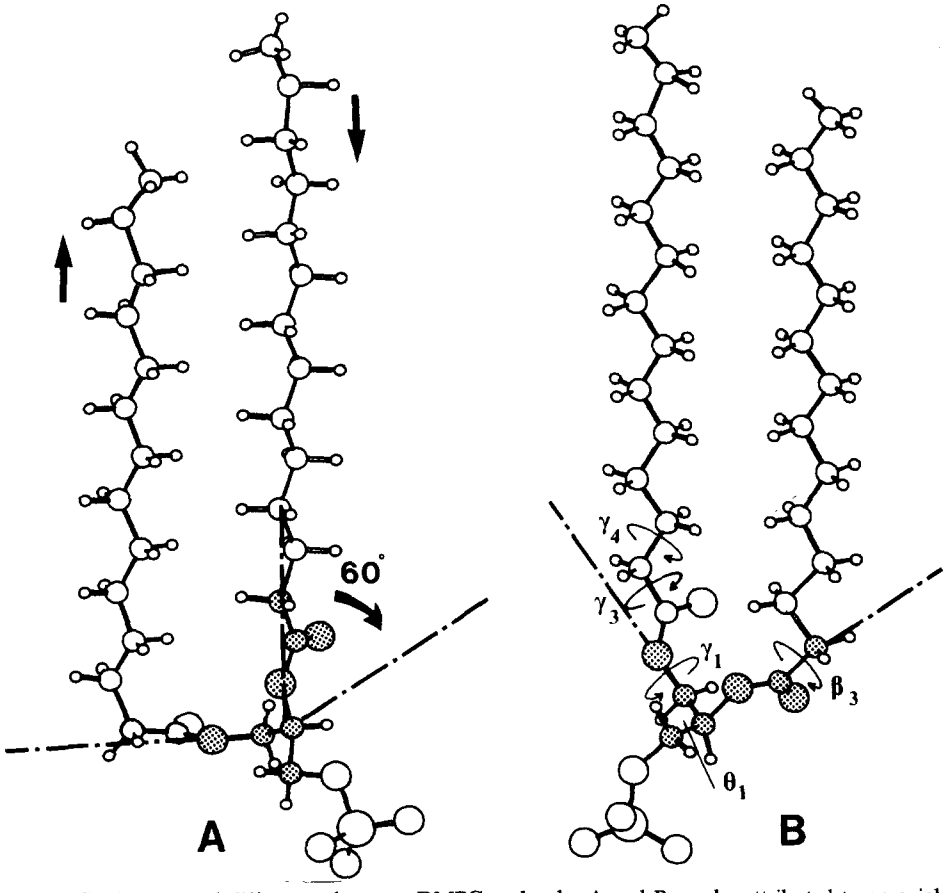


Fig. 5. Conformational differences between DMPG molecules A and B can be attributed to an axial displacement of the fatty acid chains (arrows) and a reorientation of the central diacylglycerol part (dotted atoms). The fatty acids thereby change their conformation from straight to bent and vice versa. The bonds involved in this conformational change are indicated (rotation arrows). The glycerol headgroup has been omitted for clarity.

the γ-chain straightens and projects beyond the β-chain. As indicated in Fig. 5 and obvious from the torsion angles in Table II, the conformation of the central part of the diacylglycerol group (dotted atoms) is largely unaffected. Changes are confined to torsion angles $\gamma_1$, $\gamma_3$, $\gamma_4$ and $\beta_3$ (see Table II). Compared to molecule A this leads to a lateral shift of the chains (compare Fig. 4b).

*Headgroup conformation.* As shown in Figs. 2b and 4b the phosphoglycerol headgroup is turned away from the diacylglycerol part and extends parallel to the bilayer surface. This gives the molecule an L-shape. As mentioned above the headgroups of molecule A and B are mirror images both with respect to configuration and conformation. The α-torsion angles (Table II) of the headgroups of molecule A and B thus are practically identical but of opposite direction. Note, however, that molecules A and B are not related by any crystallographic symmetry and thus this mirror image conformation is not perfect.

At the glycerol bond C(1)-C(2) the pseudo mirror symmetry is discontinued. The torsion angles about this bond ($\theta_1/\theta_2$) are ap/− sc and +sc/ap in molecule A and B, respectively.

The three oxygens of the glycerol headgroup O(12), O(15) and O(16) have mutually ($\alpha_5/\alpha_6$) +sc/+sc (molecule A) or −sc/−sc conformation (molecule B). The two unsubstituted glycerol oxygens are oriented towards the layer interface and are involved in hydrogen bonds and coordination bonds (see below).

*Polar group packing and interactions*

Fig. 6 shows the arrangement and interactions of the phosphoglycerol headgroups in views parallel and perpendicular to the bilayer interface. The sodium ions form a layer that is sandwiched by phosphoglycerol groups of adjacent bilayers. The sodium ions are placed ±0.3 Å and the phosphate groups ±1.8 Å from the bilayer interface. As it can be seen in the projection onto the layer plane (Fig. 6b) the (+) and (−) charges are not distributed uniformly at the bilayer surface. The (+) sodium ions as well as the (−) phosphate groups are aligned in zigzag rows extending in *a*-direction. In *b*-direction rows of phosphate groups alternate with rows of sodium ions.

The glycerol groups project alternatingly to the left and to the right from the phosphate rows. Laterally, the headgroups interact by an extensive network of hydrogen bonds, ionic bonds and coordination bonds.

A sequence of two hydrogen bonds (2.8 and 2.7

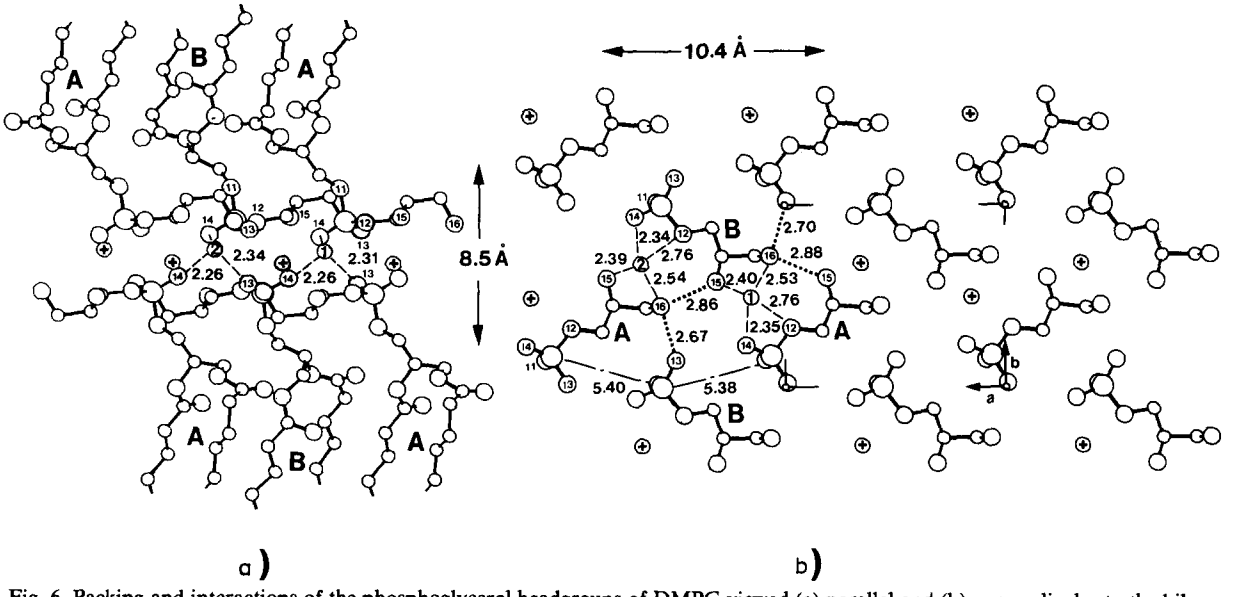


Fig. 6. Packing and interactions of the phosphoglycerol headgroups of DMPG viewed (a) parallel and (b) perpendicular to the bilayer interface. Hydrogen bonds (dotted lines) and ionic and coordination bonds (borken lines) are indicated. The contact distances are given in Å.

Å) starts at the glycerol hydroxyl oxygen O(15) of molecule A. The bonds are directed via hydroxyl oxygen O(16) of molecule B and terminated at a phosphate oxygen O(14) of another A molecule. A corresponding sequence of bonds, but with opposite orientation in the bilayer plane, runs between molecules B–A–B.

The sodium ions form bond contacts both laterally and across the bilayer interface. Each sodium ion is coordinated by six oxygen atoms. Three of these contacts are formed with unsubstituted (−) charged phosphate oxygens and have the character of short ionic bonds (< 2.40 Å). The other three contacts are established with formally neutral oxygens (one esterfied phosphate oxygen O(12) and two unsubstituted glycerol oxygens O(15), O(16)) and may be considered as co-ordination bonds. The $Na^+ \cdots O$ contact distance of these three latter bonds is somewhat longer (2.40–2.76 Å) than those of the ionic bonds. Differences in bond lengths appear to reflect the degree of negative polarisation of the oxygen atoms. The rather short coordination bond between $Na^+ \cdots O(15)$ thus might be due to the fact, that this hydroxyl oxygen serves as a proton donator only and becomes more polarized than O(16) which acts both as a donor and acceptor.

## Discussion and Conclusions

The present crystal analysis provides important information on the structural organization of DMPG and of membrane lipids in general. In particular with respect to conformation of the hydrophobic diacylglycerol moiety, the chain packing mode and the headgroup packing the DMPG structure reveals a variety of new interesting features. Moreover the structural parameters of DMPG closely resemble the parameters recorded for the gel phase of DPPG indicating that the structure observed in crystals is largely preserved on hydration.

### *Preferred conformations*

Regarding the conformation of the diacylglycerol part, the DMPG structure now confirms earlier observations, that the fatty acid carrying glycerol oxygens O(21) and O(31) can mutually adopt both a +sc and a −sc conformation about the C(2)-C(3) glycerol bond (torsion angle $\theta_4$) [21,25,26]. The $\theta_4 = +sc$ conformation is the most predominant one and has been found in a great number of crystal structures of both double chain [23,26–28] and single chain (lyso)glycerophospholipids [29–31].

The $\theta_4 = -sc$ conformation has so far only been observed in three glycerolipids, in dilauroylphosphatidyldimethylethanolamine [25], in diacylglycero-*p*-toluenesulphonate (a model lipid) [32] and now in DMPG.

Moreover, for lipids with the $\theta_4 = +sc$ confirmation it could be shown that the glycerol back bone can change its orientation towards the bilayer plane from layer perpendicular to layer-parallel and vice versa by an axial displacement of the hydrocarbon chains [25,31]. The present DMPG structure shows that a similar axial displacement of the hydrocarbon chains and a reorientation of the glycerol back bone also occurs for the lipids with the $\theta_4 = -sc$ conformation. Thus we can now distinguish four minimum energy conformations for the diacylglycerol group which arise from two simple motions of the hydrocarbon chains:

(1) an axial ±120° rotation of the hydrocarbon chain about the C(2)-C(3) glycerol bond giving rise to the +sc and −sc conformation of the two fatty acid carrying glycerol oxygens [25] and

(2) an axial displacement of the chains producing a reorientation of the glycerol back bone and a shift of the chain bend from the β-fatty acid to the γ-fatty acid and vice versa (compare Fig. 5 and Ref. 28).

According to NMR-studies these kind of rotational and translational motions of the chains are actually the fundamental motions that occur in the diacylglycerol part in the liquid-crystalline state [33] and the described minimum energy conformations co-exist in the bilayer in a dynamic equilibrium (Ref. 34, and Hauser, Pascher and Sundell, unpublished data).

### *Hybride subcells*

With regard to the lateral chain packing, the subcell of DMPG (Fig. 3) and other complex membrane lipids [23,24] show that simple packing modes, such as $O_\perp$, $M_{||}$ and $T_{||}$ [22] can combine to a variety of hybride packing modes. These

hybride packing modes have to be considered as intermediate states between the well-ordered, simple chain packing modes and the less ordered hexagonal chain matrix [35].

The great variability of the chain packing modes apparently can give rise to statistical disorder in the chain matrices of multilamellar lipid crystals and thus lead to complications in the solution of single crystal structures. Furthermore, these hybride packings obstruct the interpretation of the chain arrangement from high-angle reflexions (short spacings) of X-ray powder diffractions and from infrared and Raman spectra. Little or no information is at present available on the interpretation of hybrid packing modes by the above mentioned techniques.

*Lateral packing of the headgroup*

The packing pattern of the phosphoglycerol headgroup (Fig. 6) displays far reaching similarities with that of the phosphocholine headgroup [21]. In both headgroup arrangements the (−) phosphate groups and the (+) sodium ions or choline groups, respectively are aligned in separate rows. Similarly to the glycerol groups the choline groups extend to the left and right from these phosphate rows.

There is however a significant dissimilarity. The phosphate groups of PC are linked to rows by water molecules of hydration. In PG there is no such direct interactions. A contact between the phosphate groups is mediated by the distal glycerol-hydroxyl group O(16). Nevertheless the phosphate-phosphate distances and packing geometry is almost identical. In the PC pattern the distance between the phosphate rows (*b*-axis) is 1 Å longer than in the PG structure. This difference is due to the longer ionic bond contacts between the phosphate group and the bulky quarternary ammonium groups (3.7 Å) compared to the phosphate-sodium contacts (2.4 Å).

*Ressemblance of the PG crystal structure and the gel phase*

From X-ray diffraction studies of aqueous dispersions of DPPG, Watts et al. [10] have calculated the bilayer-thickness $d_1$, molecular area $S$, chain tilt $\phi$ and partial specific volume $V_1$ of the gel phase at pH 8 (see Table III). Based on the crystal structure of DMPG and with consideration of the increased chain length of the dipalmitoyl homologue the corresponding structural parameters for the anhydrous crystal phase of DPPG can be calculated. As shown in Table III the main difference between gel and crystal phase concerns the partial specific volume. The value $V_1 = 1.01$ ml · g$^{-1}$ recorded for the gel state no doubt, appears to be very large compared to that of the crystal phase $V_1 = 0.889$ ml · g$^{-1}$.

The determination of $V_1$ in aqueous dispersions, however, is a rather troublesome task. Any error in $V_1$ also affects the value of the molecular area and of the chain tilt. Assuming that $V_1$ is 0.91 instead of 1.01 ml · g$^{-1}$, the molecular area of the gel phase is reduced from 48 to 44 Å$^2$, the packing cross-section of the crystal phase. The minor difference in bilayer thickness then can be attributed to the less effective chain packing and thus to a diminished chain tilt (25° instead of 29°).

Regardless of possible experimental errors the structural parameters of the gel phase and the crystal phase are similar to an extent indicating that the structural features observed in the anhydrous crystal are largely preserved also in the hydrated gel state.

TABLE III

STRUCTURAL PARAMETERS

Comparison of structural parameters of DPPG in the gel phase at pH 8 [10] and in the anhydrous crystal phase (calculated from the DMPG structure).

| | $d_1$(Å) | $S$(Å) | $\phi$(°) | $\bar{V}_1$(ml·g$^{-1}$) |
|---|---|---|---|---|
| DPPG gel phase | 51.1 | 48.0 | 32 | 1.01 |
| DPPG crystal phase | 50.0 | 44.0 | 29 | 0.889 |

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