References
Beato, M., & Feigelson, P. (1972) *J. Biol. Chem.* 247, 7890-7896.
Cake, M. H., Disorbo, D. M., & Litwack, G. (1978) *J. Biol. Chem.* 253, 4886-4891.
Carlstedt-Duke, J., Gustafsson, J., & Wrange, O. (1977) *Biochim. Biophys. Acta 497*, 507-524.
Carlstedt-Duke, J., Wrange, O., Dahlberg, E., Gustafsson, J., & Hogberg, B. (1979) *J. Biol. Chem. 254*, 1537-1539.
Cidlowski, J. A., & Munck, A. (1978) *Biochim. Biophys. Acta 543*, 545-555.
Cidlowski, J. A., & Thanassi, J. W. (1978) *Biochem. Biophys. Res. Commun. 82*, 1140-1146.
Cidlowski, J. A., & Thanassi, J. W. (1979) *Biochemistry 18*, 2378-2384.
Climent, F., Bugary, H., & Beato, M. (1976) *FEBS Lett. 66*, 317-321.
Govindan, M. V., & Sekeris, C. E. (1978) *Eur. J. Biochem. 89*, 95-104.
Kalimi, M., Colman, P., & Feigelson, P. (1975) *J. Biol. Chem. 250*, 1080-1086.
Koblinsky, M., Beato, M., Kalimi, M., & Feigelson, P. (1972) *J. Biol. Chem. 247*, 7897-7904.
Martin, R. G., & Ames, B. N. (1961) *J. Biol. Chem. 236*, 1372-1379.
Middlebrook, J. L., & Aronow, L. (1977) *Endocrinology 100*, 271-282.
Muldoon T. G., & Cidlowski, J. A. (1980) *J. Biol. Chem. 255*, 3100-3107.
Munck, A. (1968) *J. Biol. Chem. 243*, 1039-1042.
Munck, A., & Leung, K. (1977) *Mod. Pharmacol.-Toxicol. 8*, 311-397.
Nishigori, H., & Toft, D. (1979) *J. Biol. Chem. 254*, 9155-9161.
O'Brien, J. M., & Cidlowski, J. A. (1980) *J. Steroid Biochem.* (in press).
Schrader, W. T., & O'Malley, B. W. (1972) *J. Biol. Chem. 247*, 51-59.
Sherman, M. R., Atienza, S. B. P., Shansky, J. R., & Hoffman, L. M. (1974) *J. Biol. Chem. 249*, 5351-5363.
Sherman, M. R., Tuazon, F. B., Diaz, S. C., & Miller, L. K. (1976) *Biochemistry 15*, 980-989.
Sherman, M. R., Pickering, L. A., Rollwagen, F. M., & Miller, L. K. (1978) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 37*, 167-173.
Vedeckis, W. V., Schrader, W. T., & O'Malley, B. W. (1978) *Biochem. Actions Horm. 5*, 321.
Vedeckis, W. V., Schrader, W. T., & O'Malley, B. W. (1980) *Biochemistry 19*, 343-349.
Wilson, E. M., & French, F. S. (1979) *J. Biol. Chem. 254*, 6310-6319.
Wrange, O., Carlstedt-Duke, J., & Gustafsson, J. (1979) *J. Biol. Chem. 254*, 9284-9290.
Yamamoto, E. R., & Alberts, B. M. (1976) *Annu. Rev. Biochem. 45*, 721-746.

Conformation of Phosphatidylethanolamine in the Gel Phase As Seen by Neutron Diffraction†

Georg Büldt* and Joachim Seelig

ABSTRACT: For confirmation of some general aspects of phospholipid conformation in membranes and extension of previous neutron diffraction studies on dipalmitoyllecithin, measurements have now been made on 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE) in the gel phase by the same method. Three selectively deuterated samples were investigated; in one of the specimens the first methylene segment close to the glycerol backbone in both chains was deuterated, and in the other two samples one of the methylene segments in the phosphoethanolamine group was replaced by CD_2. Together with the undeuterated DPPE, these probes were investigated at very low water content (about 1.5-2 molecules of water per lipid) as oriented samples at 25 °C. The intensities of the first 12 reflections were collected and phased, and the mean positions of the segments were determined. The results confirm the idea that the conformation of a DPPE molecule in the gel state is very similar to the crystal structure of *rac*-1,2-dilauroyl-*sn*-glycero-3-phosphoethanolamine. The two main features are (1) the chains remain in all all-trans conformation having an axial displacement of about 3-4 Å, (2) the zwitterionic dipoles in the head groups of both compounds are found to be aligned almost parallel to the bilayer surface. The main advantage of the method results in the fact that the combination of neutron scattering with selectively deuterated probes allows the determination of the mean label position to an accuracy of up to ±1 Å.

When the characteristics of the phospholipids in biological membranes were investigated, it was soon found that many lipids in their pure form exist in several thermodynamic phases, dependent on water content, temperature, pH, and ion concentration (Chapman et al., 1967; Luzzati, 1968; Chapman & Wallach, 1968; Sackmann, 1978). The main structural features of these phases were determined by X-ray diffraction (Tardieu et al., 1973; Levine & Wilkins, 1971; Janiak et al., 1976). At least two of these phases seemed to be particularly important for biological membranes, the gel phase and the liquid-crystalline phase. Moreover, it was found that changes of temperature, pH, or ion concentration can cause a transition between these two states (Träuble & Eibl, 1974). The effect

† From the Department of Biophysical Chemistry, Biocenter of the University of Basel, CH-4056 Basel, Switzerland. *Received March 6, 1980.* This work was supported by Grant 3.409.78 from the Swiss National Science Foundation and by a European Molecular Biology Organization travel grant (G.B.).

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of these phases on the function of integral membrane proteins and on their distribution in the plane of the membrane has been well demonstrated in reconstituted systems of membrane proteins with only one kind of lipid (Kleemann & Mc Connell, 1976) and for natural membranes enriched in one lipid species (Overath et al., 1970; Wallace et al., 1976).

Thus it was important to look for methods that could give a detailed structural picture about the lipid conformation in the head group and glycerol backbone region as well as values which can characterize the statistics of the hydrocarbon chain conformation. One type of method that has been very successfully used is nuclear magnetic resonance, especially deuterium magnetic resonance (^{2}H NMR) (Seelig, 1977; Oldfield et al., 1978; Stockton et al., 1977; Seelig & Seelig, 1978). From these experiments, the second moment of the segmental orientation function is obtained; this, in terms of order parameter profiles, has been proved to be a very sensitive property of the chain conformations.

On the other hand, positional information as obtained from X-ray and neutron investigations gives direct access to these features. Here different approaches have been made in the past. One possibility is small-angle scattering on unilamellar vesicles or membrane dispersions. As was first shown by Wilkins et al. (1971), Engelman (1971), Lesslauer et al. (1972), and Pape et al. (1974), these experiments can also give information about the bilayer profile but, unfortunately, with low resolution. The other possibility is diffraction on multilamellar stacks of membranes where resolution is increased more according to the enhancement of the intensity by the one-dimensional lattice factor, but here also profile resolution does not exceed (4–5 Å in the best cases [see, e.g., Lesslauer et al. (1972); Torbet & Wilkins, 1976]. It has been shown, however, that by neutron diffraction using selectively deuterated lipids it is possible to determine the mean position of the labeled segment with a precision of ±1 Å in many cases (Büldt et al., 1978, 1979; Zaccai et al., 1979). Here the information is obtained by subtracting the structure factors of a sample deuterated in a certain position from the structure factors of the undeuterated sample.

In the present paper we continue this work with respect to 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE) in the gel phase. This seemed to us another interesting phospholipid with similar overall properties as 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) so that the results together with those of DPPC could give rise to some more general ideas about the conformation of phospholipids in membranes. In addition, *rac*-1,2-dilauroyl-*sn*-glycero-3-phosphoethanolamine (DLPE), which is four methylene segments shorter than DPPE, is one of the rare examples of a phospholipid which has been crystallized (Hitchcock et al., 1974). Very recently crystals from 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) have also been grown and their structures determined (Pearson & Pascher, 1979). It is clear that the structure of a phospholipid in a crystal cannot provide a picture of the chain statistics (especially not for a lipid in the fluid phase), but it may provide a firm basis for the discussion of those regions in the lipids which seem to be less flexible such as the glycerol backbone. Thus it is interesting to notice that in both crystals the glycerol part and the *sn*-1 fatty acyl chain are extended nearly in one line whereas the *sn*-2 chain has a sharp bend at the beginning which results in an axial displacement of about 3.6 Å between the two chains. Another important feature seen in the crystals is the orientation of the zwitterionic dipole which is almost parallel to the bilayer surface. As we were able to show in our previous work, these basic characteristics derived from the crystal data are still preserved in DPPC when it forms bilayers (Büldt et al., 1977, 1979; Zaccai et al., 1979).

For the present paper, we shall investigate these features for the gel state of DPPE. For this purpose, we synthesized three different samples of DPPE deuterated in the C_β position (NCD_2CH_2P) and the C_α position (NCH_2CD_2P) of the phosphoethanolamine group. Another sample was deuterated in the C-2 positions [$CCD_2(CH_2)_{13}CH_3$] of both fatty acyl chains; the CD_2 segment in the chain which is attached to the first carbon atom of the glycerol backbone is called the C-2(1) label whereas the CD_2 segment in the other chain is the C-2(2) label.

Materials and Methods

The synthesis of the samples deuterated at the C_β, C_α, and C-2 positions has been described in detail by Gally et al. (1975), Seelig & Gally (1976), and Seelig & Browning (1978). Undeuterated DPPE was purchased from Fluka. The purity of all samples was checked before and after the experiment by thin-layer chromatography.

The preparation of oriented multilayers was achieved as follows: Forty milligrams of each sample was sonicated in 1 mL of ethanol and then spread on a very clean quartz slide (6.5 × 2.5 cm^2). The ethanol was slowly evaporated by a gentle stream of nitrogen. Residual ethanol was removed by placing the slides into high vacuum for 12 h.

The quality of the orientation of these multilayer stacks was tested by X-ray measurements on these slides to obtain high enough resolution in the neutron experiments. They were inserted in a double-mirror focussing camera. Each slide was slowly rotated by a motor (1° in 6 min) about a horizontal axis which was aligned to go parallel to the slide through the thin lipid film. The sample was then further aligned so that this axis of rotation crossed the X-ray beam at right angles. The longer extension of the cross sectional area of the X-ray beam (8 × 1 mm at the sample position) is thus oriented parallel to the axis of rotation. A photographic film mounted at the position at the point focus of the camera showed the arcs of the lamellar diffraction. The intensity distribution on the arcs was a measure of the mosaic spread of the sample. The slide was oscillated within a certain angular range several times in order to see how many reflections could be collected in a resonable time.

The neutron diffraction experiments were carried out on a 4-circle diffractimeter, the D16 instrument at the Institut Laue-Langevin (ILL) in Grenoble. The experimental setup, the data acquisition, and the corrections for the integrated intensities have been described in detail by Büldt et al. (1979). The diffraction patterns of the samples were measured by θ–2θ scans at 25 °C and 100% relative humidity. The samples were studied with different amounts of H_2O/D_2O mixtures in the water layer between the bilayers, the undeuterated sample and the C-2 sample in H_2O, 50% D_2O, and D_2O, the C_β sample in H_2O and D_2O, and the C_α sample in H_2O. From the changes in the profiles going from H_2O to D_2O, it was seen that very little water was trapped between the layers; this was estimated to be about 1.5–2 molecules of water/lipid.

Results

In former experiments (Büldt et al., 1979) the water was used as an isomorphous replacement site for phasing the reflections. In the present case, since the water uptake is low, only those reflections that strongly depend on H_2O/D_2O ratio could be phased by this means as indicated in Table I.

Table I: Experimental Structure Factors of the Lamellar Reflections from Three Oriented Samples in H_2O (Lamellar Spacing 55.8 Å)[a]

h	DPPE	C_β	C-2
1	−42.3 ± 4.0 w	−58.8 ± 4.0 w	−49.1 ± 4.0 w
2	18.2 ± 2.0 w	33.8 ± 2.0 w	9.2 ± 2.0 w
3	0.0 ± 1.5	−11.9 ± 1.5 w	19.3 ± 1.5 w
4	−26.6 ± 1.5 w	−20.5 ± 1.5 w	−34.5 ± 1.5 w
5	10.1 ± 1.5 w	7.8 ± 1.5 w	7.5 ± 1.5 w
6	−5.4 ± 1.3 w	−7.1 ± 1.3 w	−6.8 ± 1.3 w
7	−13.9 ± 1.0	−9.8 ± 1.2	−21.3 ± 1.3
8	9.0 ± 1.2	0.0 ± 1.2	11.4 ± 1.2
9	0.0 ± 2.0	0.0 ± 2.0	0.0 ± 2.0
10	0.0 ± 3.0	−18.5 ± 3.0 w	16.5 ± 3.0 w
11	16.1 ± 4.0	23.7 ± 4.0	
12	−30.1 ± 7.0	−35.5 ± 7.0	

[a] "w" indicates that these phases were determined by using the water layer as an isomorphous replacement site. The uncertainties of these structure factors are partly the standard deviations of the neutron counts. In addition, the uncertainties of the first three structure factors were also influenced by an estimated error from the vertical slit correction factor. Because the orders 11 and 12 are obtained against a steeply sloping background arising from quartz scattering, the errors are relatively larger than the others.

Therefore additionally using the classic crystallographic method, we looked for the position of the deuterated segment in the difference Patterson map

$$P(x) = \frac{2}{d}\sum_{h=1}^{h_{max}}[|F^o_D(h)| - |F^o_H(h)|]^2 \cos 2\pi xh/d \quad (1)$$

where d is the lamellar repeat distance and $F^o_D(h)$ and $F^o_H(h)$ are the observed structure factors with the deuterated and the undeuterated segment, respectively. This Patterson map will become incorrect if several phases change between corresponding structure factors in both sets of data. Therefore this procedure can only give correct solutions in cases where the scattering length of the label is not too strong as for C_β and C_α labels, where only one CD_2 segment is introduced into the head group. Experimentally it is possible to detect a phase change between the deuterated and undeuterated sample by preparing a specimen which is, for example, a 1:1 mixture of these lipids. In a plot of the corresponding structure factors against the lipid fraction of the mixture a phase change would be detected. The scaling factors between the samples can be calculated from the structure factors of the water layer. At low water content, however, these structure factors are not very precise and can introduce large uncertainties in the values of the scaling factors. In addition, since the amount of material available was low, we could not use this procedure. In practice, instead of using only the one-dimensional difference Patterson map, a simple model for the shape of the deuterated segment in projection to the bilayer normal was introduced, and the position and the scaling factor was obtained from a fit of the absolute value of the calculated structure factors $|F^c(h)|$ to the difference of the absolute values of the observed structure factors $|F^o_D(h)|$ and $|F^o_H(h)|$. As in our earlier work, a Gaussian-shaped model was assumed

$$g(x) = \frac{1}{\mu(2\pi)^{1/2}}\left[\exp\left(-\frac{(x-x_0)^2}{2\mu^2}\right) + \exp\left(-\frac{(x+x_0)^2}{2\mu^2}\right)\right] \quad (2)$$

where the parameter μ is the square root of the mean-square displacement of the deuterated segment and x_0 is the mean position of the segment (μ in eq 2 is slightly different from

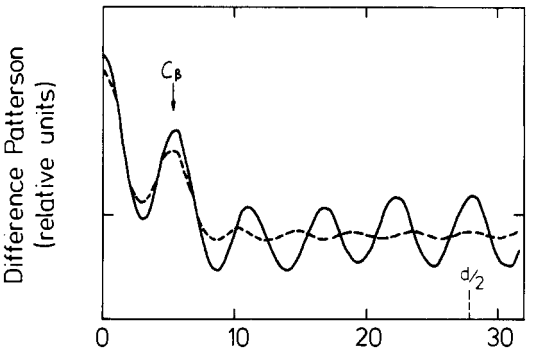

FIGURE 1: The solid line is the difference Patterson profile as obtained from eq 1 by using the unphased 12 structure factors of the C_β-labeled DPPE and of the undeuterated DPPE; the broken line is the Patterson plot determined from 12 structure factors (eq 3) calculated for a Gaussian label (eq 2) with the parameters x_0 = 25.4 Å and μ = 0.9 Å.

ν as defined in previous papers, $\nu^2 = 2\mu^2$). The calculated structure factors for this model are

$$F^c(h) = 2t \exp[-2(\mu\pi h/d)^2] \cos(2\pi x_0 h/d) \quad (3)$$

where t is the scattering length of the label. This leads to the minimization procedure

$$[||F^o_D(h)|s - |F^o_H(h)|| - |F^c(h)||]^2 \rightarrow \min \quad (4)$$

where s is the scaling factor, which is necessary since $F^o_D(h)$ and $F^o_H(h)$ are obtained from different slides. From this minimization procedure, the following values for the C_β position were obtained:

sample in H_2O: x_0 = 25.4 Å; μ = 0.9 Å

sample in D_2O: x_0 = 25.6 Å; μ = 1.2 Å

The distance x_0 is measured from the center of the hydrocarbon chain region. The x_0 values for the samples in H_2O and D_2O are very close together although they are derived from two independently measured sets of reflections, which proves that these data are very reliable. Figure 1 shows the difference Patterson map of the observed structure factors (solid line) and the calculated Patterson profile of the Gaussian label (broken line) using the parameters determined in the above fit. The peak at 5.4 Å in the difference Patterson map corresponds to a mean position of the C_β label of x_0 = 25.2 Å. Thus these preliminary results give a certain value of x_0 so that this segment can now be used as an isomorphous replacement site to determine the signs of the structure factors. When a Hargreaves plot was used (Zaccai et al., 1975), those phases for the deuterated and the undeuterated samples were found which could not be determined from the water layer (Table I). By fitting now the $F^c(h)$ values to the phased structure factors with

$$[F^o_D(h)s - F^o_H(h) - F^c(h)]^2 \rightarrow \min \quad (5)$$

we obtained the same x_0 and μ values as given above. For the C_α-labeled sample reflections up to the 12th order were collected in H_2O. Unfortunately the reflections 9 and 10 were lost by an accident in the machine computer so that a difference Patterson plot cannot be given. However, a fit according to eq 4 is still possible and gives for the C_α segment x_0 = 25.4 Å and μ = 1.1 Å, which together with the position of the C_β segment determined above is a first indication that the dipole orientation is parallel to the membrane surface.

In the case of the C-2 labels, only the first 10 of 12 observable orders were used since reflections 11 and 12 are much weaker then in the case of the C_β label. As seen from Table I, these orders have large errors which result from the fact that

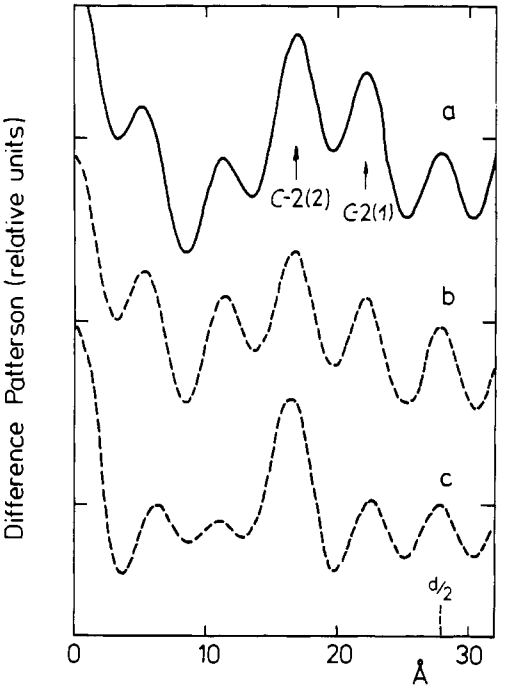

FIGURE 2: (a) Difference Patterson according to eq 1 by using the first 10 structure factors of the C-2 labeled DPPE and the unlabeled DPPE. The arrows mark the two peaks which correspond to the C-2(1) and C-2(2) label. (b) Patterson plot determined from 10 structure factors calculated for two Gaussian labels with the parameters x_0 = 17.6 Å for the C-2(1) label and x_0 = 22.0 Å for the C-2(2) label and μ = 0.3 Å in both cases. (c) Patterson plot as in (b) but with the same value of x_0 = 19.8 Å for both labels and μ = 0.3 Å.

they are subtracted from the strongly increasing background of the quartz scattering at these angles. The difference Patterson was plotted as shown in Figure 2a to obtain an indication of the positions of the C-2 labels. Two peaks are seen in this figure, showing the label positions of C-2(1) at 16.7 Å and of C-2(2) at 19.5 Å. It is clear that these values are influenced by Fourier truncation errors. Therefore a fit according to eq 4 was made, and the positions were found to be 17.6 Å for C-2(1) and 22.0 Å for C-2(2). The Patterson diagram corresponding to these values is shown in Figure 2b, having two peaks at the same positions as in 2a. For comparison, the lower plot (Figure 2c) gives the Patterson map for two Gaussian labels which have the same position in the profile; the parameters are x_0 = 19.8 Å and μ = 0.3 Å. Here only one dominant peak occurs. Thus these plots indicate that both chains are out of step by as much as 3 Å. This, however, should not be considered as a proof but as a first indication since phase changes between corresponding structure factors might alter this picture. In the next step, the mean positions of the C-2(1) and C-2(2) labels were determined from a fit according to the minimization condition (eq 5) using only those structure factors of the C-2 sample (Table I) where the phases have already been determined by using the water layer as an isomorphous replacement site. The values obtained were 17.5 Å for C-2(1) and 21.9 Å for C-2(2). Then in order to determine the phases of the 7th and 8th orders of the C-2 sample, all phase combinations of these structure factors were added to the already phased reflections and used in the fit (eq 5) with the additional constraint for the two x_0 values to deviate not more than ±2 Å from the predetermined values 17.5 and 21.9 Å. The phases for reflections 7 and 8 were found as given in Table I and the following final mean positions were obtained: x_0 = 17.4 Å for C-2(1) and x_0 = 21.1 Å for C-2(2). Thus also in this case the phases of the structure factors were determined by using the water layer and the label as isomorphous replacement sites. Figure 3 shows in (a) the profile of the C-2 deuterated sample together with the undeuterated DPPE in (b) for the first 10 orders. The difference between these profiles is seen in (c). Two partly overlapping peaks clearly arise over the noise.

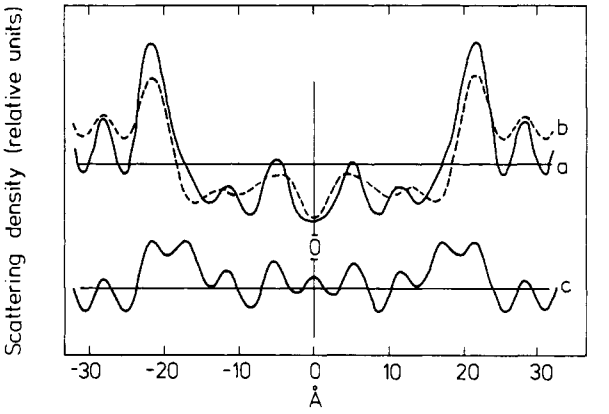

FIGURE 3: Neutron scattering length density profiles calculated from 10 structure factors of (a) DPPE deuterated at the C-2 position, (b) of the undeuterated sample, and (c) the difference of these profiles.

Discussion

A more detailed comparison of our results with the crystal structure of DLPE (Hitchcock et al., 1974; Elder et al., 1977) can now be given. If four additional CH_2 groups are added to each chain in this structure, an expanded unit cell for DPPE (a = 47.7 Å for DLPE) is found, which is in agreement with the lamellar spacing of 55.8 Å obtained from the present neutron data of DPPE. This shows the overall similarity of the structures and gives an indication that the chains in the bilayer are in an extended all-trans conformation. This modified crystal structure was used as a model together with the atomic coordinates and their temperature factors as given by Elder et al. (1977) to calculate the first 12 structure factors for the neutron profile given in Figure 4a. Then from a space-filling model, the coordinates of the atoms in the phosphoethanolamine group were determined when it was oriented perpendicular to the membrane surface. The corresponding neutron profile is given by curve b in Figure 4. Thus as can be seen, no measurable differences can be obtained by neutron diffraction from undeuterated samples between these two conformations. Now, in the C_β position, the CH_2 group was replaced by a CD_2 group, and the profiles were calculated to the same resolution, in Figure 4c with the phosphoethanolamine group as in the crystal and in Figure 4d with this group perpendicular to the bilayer surface. A big difference between the two conformations calculated is observed.

These profiles are now compared with the experimental results. By using the structure factors from the C_β-labeled DPPE and from the undeuterated sample, corresponding profiles are plotted in Figure 5a and b; the difference of these profiles is shown in Figure 5c. Parts d and e of Figure 5 are the differences of the profiles (c) minus (a) and (d) minus (b) of Figure 4, respectively. These profiles show that the conformation of the head group found in the crystal of DLPE is approximately preserved in the gel-phase structure of DPPE. The small difference between the peak positions in curves c and d of Figure 5 may indicate that in this phase the dipole is oriented by a small angle out of the plane of the bilayer surface. This conformation is probably the result of a network of hydrogen bonds as seen in the crystal structure and elec-

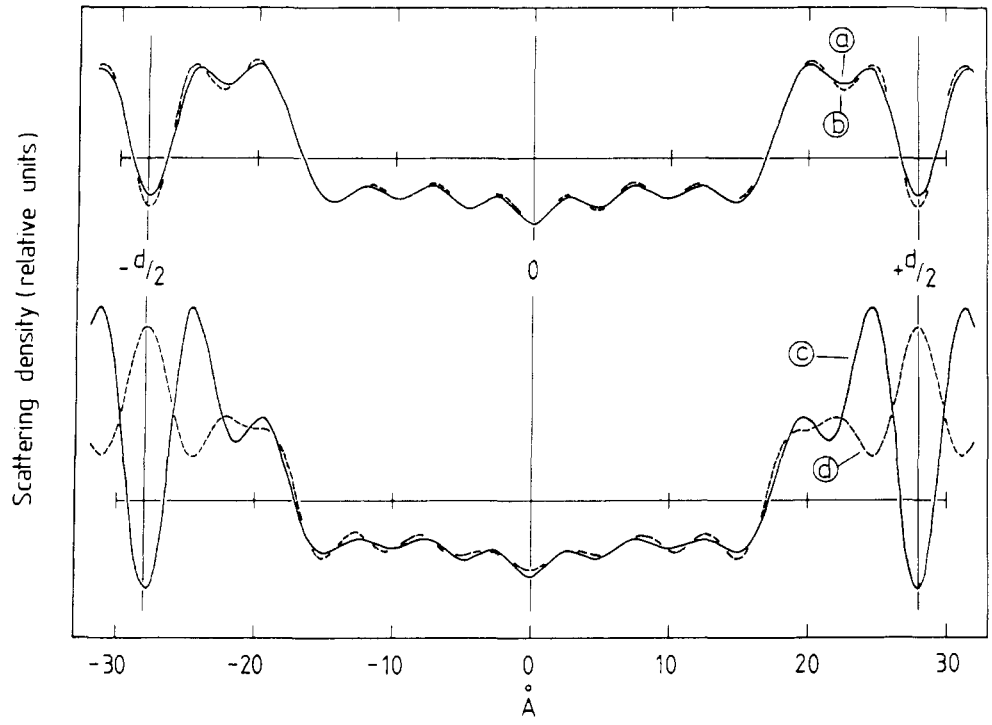

FIGURE 4: The DLPE molecule as found in the crystal structure is lengthened by four methylene segments in each chain to obtain a model structure for DPPE. When the coordinates and temperature factors as given by Elder et al. (1977) are used, the neutron scattering length density profiles corresponding to the first 12 structure factors are plotted in (a) for undeuterated DPPE and in (c) for DPPE deuterated at the C_β position. Then from a space-filling model, the coordinates of the same structure but with the phosphoethanolamine group perpendicular to the membrane surface were derived. The corresponding profiles up to the same resolution are given in (b) for the undeuterated and in (d) for the structure deuterated at the C_β segment.

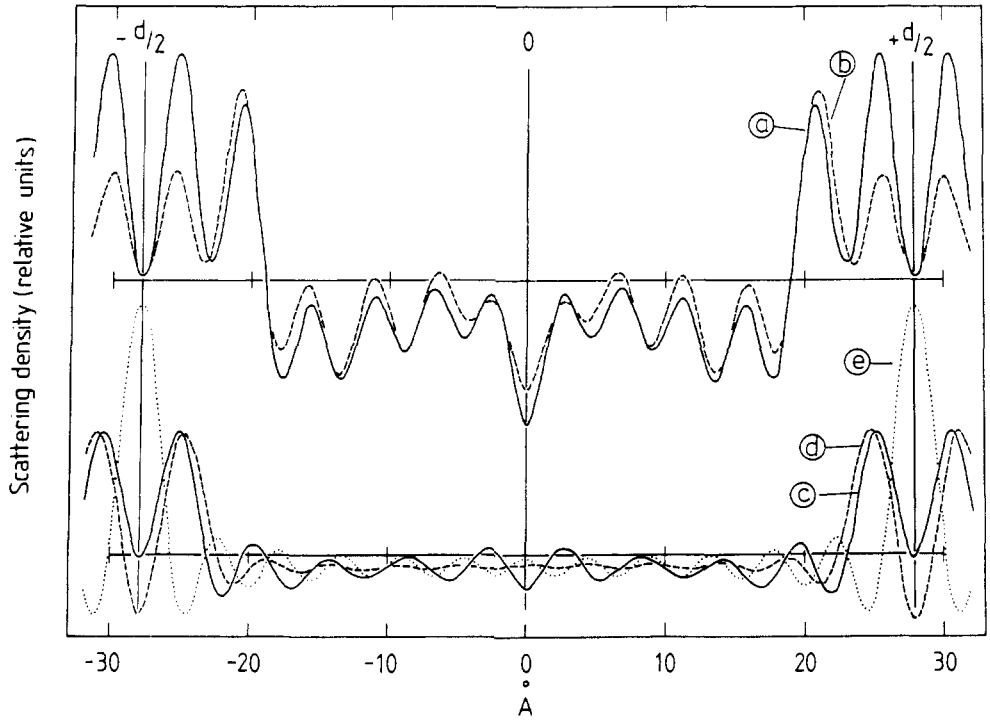

FIGURE 5: Neutron scattering length density profiles determined from 12 structure factors of (a) DPPE deuterated at the C_β position and (b) the undeuterated sample, and (c) is the difference of these profiles. The curves in (d) and (e) correspond to the differences of the calculated profiles in Figure 4. In (d) [which is (c) minus (a) of Figure 4], the phosphoethanolamine group is oriented as in the crystal almost parallel to the bilayer surface, and, in (e) [which is (d) minus (b) of Figure 4], the dipoles are perpendicular to the bilayer surface.

trostatic interactions between charges of neighboring lipid-head groups in the plane of the membrane, but this conformation may also be affected by interactions with head groups of the next layer. In the similar case of DPPC, this point has been investigated to some extent by increasing the water content between the layers from 6 wt % to 25 wt % (Büldt et al., 1978, 1979). No measurable change in the mean orientation of the dipoles was found, although the water layer increases by 6.5 Å in thickness.

The C-2 results, as seen in Figure 3, suggest that the longitudinal displacement of the fatty acid chains in phospholipids as first observed for the crystals of DLPE may be

a general conformation also for the other phospholipids. Its existence has now been directly proved by neutron diffraction for the gel state of DPPE and DPPC and more indirectly shown by ^2H NMR for the liquid-crystalline state for DPPC, DPPE, POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine), and DPPS (1,2-dipalmitoyl-*sn*-glycero-3-phosphoserine) in model membrane systems (Seelig & Browning, 1978) and for the elaidate-enriched membranes of *Escherichia coli* (Gally et al., 1979).

In the ^2H NMR experiment, a difference between the quadrupolar splitting of the C-2 segments in the chains is seen which is interpreted as the consequence of the sharp-bend conformation at the beginning of the *sn*-2 chain whereas the *sn*-1 chain continues more in the direction given by the glycerol backbone. This finally results in the axially displacement between the chains. These findings further indicate that the conformations of the glycerol backbone and the glycerol–fatty acid ester bonds are very similar, as in the crystal. The other general feature is that the zwitterionic dipoles in the head group have a mean orientation almost parallel to the bilayer surface as also indicated by dielectric measurements (Shepherd & Büldt, 1978).

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References

Büldt, G., Gally, H. U., Seelig, A., Seelig, J., & Zaccai, G. (1978) *Nature (London) 271*, 182.

Büldt, G., Gally, H. U., Seelig, J., & Zaccai, G. (1979) *J. Mol. Biol. 134*, 673.

Chapman, D., & Wallach, D. F. H. (1968) *Biol. Membr. 1*, 125–199.

Chapman, D., Williams, R. M., & Ladbrooke, B. D. (1967) *Chem. Phys. Lipids 1*, 445.

Elder, M., Hitchcock, P., Mason, R., & Shipley, G. G. (1977) *Proc. R. Soc. London, Ser. A 354*, 157.

Engelman, D. M. (1971) *J. Mol. Biol. 58*, 153.

Gally, H. U., Niederberger, W., & Seelig, J. (1975) *Biochemistry 14*, 3647.

Gally, H. U., Pluschke, G., Overath, P., & Seelig, J. (1979) *Biochemistry 18*, 5605.

Hitchcock, P. B., Mason, R., Thomas, K. M., & Shipley, G. G. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 3036.

Janiak, M. J., Small, D. M., & Shipley, G. G. (1976) *Biochemistry 15*, 4575.

Kleeman, W., & Mc Connell, H. M. (1967) *Biochim. Biophys. Acta 419*, 206.

Lesslauer, W., Cain, J. E., & Blasie, J. K. (1972) *Proc. Natl. Acad. Sci. U.S.A. 69*, 1499.

Levine, Y. K., & Wilkins, M. H. F. (1971) *Nature (London) New Biol. 230*, 69.

Luzzati, V. (1968) *Biol. Membr., 1968 1*, 71–123.

Oldfield, E., Meadows, M., Rice, D., & Jacobs, R. (1978) *Biochemistry 17*, 2727.

Overath, P., Schairer, H. U., & Stoffel, W. (1970) *Proc. Natl. Acad. Sci. U.S.A. 67*, 605.

Pape, E. H., Menke, W., Weick, D., & Hosemann, R. (1974) *Biophys. J. 14*, 221.

Pearson, R. H., & Pascher, I. (1979) *Nature (London) 281*, 499.

Sackmann, E. (1978) *Ber. Bunsenges. Phys. Chem. 82*, 891.

Seelig, A., & Seelig, J. (1978) *Hoppe-Seyler's Z. Physiol. Chem. 359*, 1747.

Seelig, J. (1977) *Q. Rev. Biophys. 10*, 353.

Seelig, J., & Gally, H. U. (1976) *Biochemistry 15*, 5199.

Seelig, J., & Browning, L. (1978) *FEBS Lett. 92*, 41.

Shepherd, J. C. W., & Büldt, G. (1978) *Biochim. Biophys. Acta 514*, 83.

Stockton, G. W., Johnson, K. G., Butler, K., Tulloch, A. P., Boulanger, Y., Smith, I. C. P., Davis, J. H., & Bloom, M. (1977) *Nature (London) 269*, 267.

Tardieu, A., Luzzati, V., & Reman, F. C. (1973) *J. Mol. Biol. 75*, 711.

Torbet, J., & Wilkins, M. H. F. (1976) *J. Theor. Biol. 62*, 447.

Träuble, H., & Eibl, H. J. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 214.

Wallace, B. A., Richards, F. M., & Engelman, D. M. (1976) *J. Mol. Biol. 107*, 255.

Wilkins, M. H. F., Blaurock, A. E., & Engelman, D. M. (1971) *Nature (London) New Biol. 230*, 72.

Zaccai, G., Blasie, J. K., & Schoenborn, B. B. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 376.

Zaccai, G., Büldt, G., Seelig, A., & Seelig, J. (1979) *J. Mol. Biol. 134*, 693.