

## Influence of chirality on the structure of phospholipid monolayers

C. Böhm,\* H. Möhwald,\* L. Leiserowitz,† J. Als-Nielsen,§ K. Kjær§

\*Institut für Physikalische Chemie, Universität Mainz, Welder-Weg 11, 6500 Mainz, Germany

†Department of Materials and Interfaces, Weizmann Institute of Science, 76100 Rehovot, Israel

§Physics Department, Risø National Laboratory, DK-4000 Roskilde, Denmark

## INTRODUCTION

The chiral carbon centre of phospholipid molecules at the head group has a strong influence on the functional properties of these molecules in their membrane form. However, little is known about the chiral influence on the structure itself. An appropriate system for the study of such an effect on biological membranes are phospholipid monolayers on water, as the head group may assume a free energy minimum configuration in the absence of an adjacent layer. We shall address the effect of chirality on the packing properties of these monolayers by grazing incidence x-ray diffraction (GID) measurements of the chiral resolved L- $\alpha$ -dipalmitoyl-phosphatidylethanolamine (L-DPPE) and the racemic mixture DL-DPPE.

Information thereon can be derived from three-dimensional (3-D) crystals. In the crystal structure of DL-DLPE (1) the molecules form bilayers and in each layer the head groups are interlinked by NH $\cdots$ O hydrogen bonds by translation and glide symmetry. A similar packing arrangement and NH $\cdots$ O hydrogen bond network was observed in the crystal structure of phosphorylethanolamine (PEA) which can be considered as a model for the polar surface of a phospholipid bilayer (2, 3).

The phospholipid systems are extensively characterized by thermodynamic, fluorescence microscopic (4), surface potential (5) and x-ray scattering experiments (6). However to date, the contribution of the phospholipid head group to the x-ray intensity distribution of the GID data has not yet been analysed and so, little has been deduced from the GID spectrum on the head group arrangement. This is perhaps because of positional or orientational disorder of the head group.

The problem of head group ordering is also of interest from a general physical point of view: The degree of ordering in phospholipid monolayers may be similar to incommensurate or frustrated phases in three dimensional systems (7), since the spatial requirements of aliphatic tails and head groups may be different. This may eventually lead to modulated phases (8), where the tilt angle varies periodically along the surface.

## EXPERIMENTAL

L- $\alpha$ -dipalmitoylphosphatidylethanolamine (L- $\alpha$ -DPPE) and DL- $\alpha$ -DPPE were obtained from Sigma (Munich, Germany) (L- $\alpha$ -DPPE,

$\sim 99\%$ ; DL- $\alpha$ -DPPE,  $\sim 98\%$ ). The film was prepared by spreading from a chloroform/ethanol (9:1) solution (p.a., Merck) onto a millipore filtered water subphase (pH 5.5). The isotherms were exactly reproducible and the monolayers are stable over at least 24 h. In previous work, varying the chloroform/ethanol ratio, we have found that the isotherms of ethanolamines are independent of spreading solvent (4–6). Grazing incidence x-ray diffraction experiments were carried out using the liquid-surface diffractometer on beam line D4 at HASYLAB, DESY, Hamburg (Helm et al., 1987). A sealed and thermostated Langmuir trough equipped with a Wilhelmy balance was mounted on the diffractometer. The synchrotron beam was monochromated by Bragg reflection from a Ge (111) crystal to a wavelength  $\lambda = 1.38 \text{ \AA}$  and was adjusted to strike the surface at an incident angle  $\alpha = 0.85 \cdot \alpha_c$ , where  $\alpha_c$  is the critical angle for total external reflection. An elevator positions the sample at the correct height to intercept the beam. Two different linear position sensitive detectors (40 and 100 mm long) were vertically arranged and moved horizontally to measure the diffraction of the monolayers. The resolution in in-plane wave vector transfer  $q_{xy}$  amounted to  $0.025 \text{ \AA}^{-1}$ , that in normal wave vector transfer  $q_z$  to  $0.1 \text{ \AA}^{-1}$ . Measurements were performed at three different beamtimes with four film preparations. All yielded the same results.

## EXPERIMENTAL RESULTS

Fig. 1 displays in-plane diffraction scans for different  $q_z$  intervals and three different pressures below and above the break in the slope at surface pressure  $\pi_s$ , marked in the isotherm insert. At the lowest pressure (10 mN/m) the chiral resolved compound displays three peaks with maxima at 1.48, 1.45, and  $1.42 \text{ \AA}^{-1}$ , appearing most pronounced at different  $q_z$  intervals. This result was also observed for L-dimyristoylphosphatidylethanolamine (9). For the racemic mixture there are only two maxima at  $1.485 \text{ \AA}^{-1}$  and at  $1.44 \text{ \AA}^{-1}$ . (The apparent peak at  $1.36 \text{ \AA}^{-1}$  was later confirmed to be an artefact). This finding is more general for pressures below  $\pi_s$  as we will show. The peak with maximum for  $q_z \cong 0$  shifts to smaller spacings and increases in intensity with pressure as previously observed for Dimyristoyl-phosphatidylethanolamine (DMPE) (10). The width of the peak is nearly resolution limited.

A more detailed picture of the peak profiles obtained for the chiral resolved compound and the racemic mixture can be deduced from Figs. 2 and 3. At 0 mN/m and at 20 mN/m (Fig. 3) one qualitatively observes the same features as at 10 mN/m. There is one sharp intense peak for  $q_z \cong 0$  for the chiral resolved and the racemic compound, and the two types of monolayers differ in the

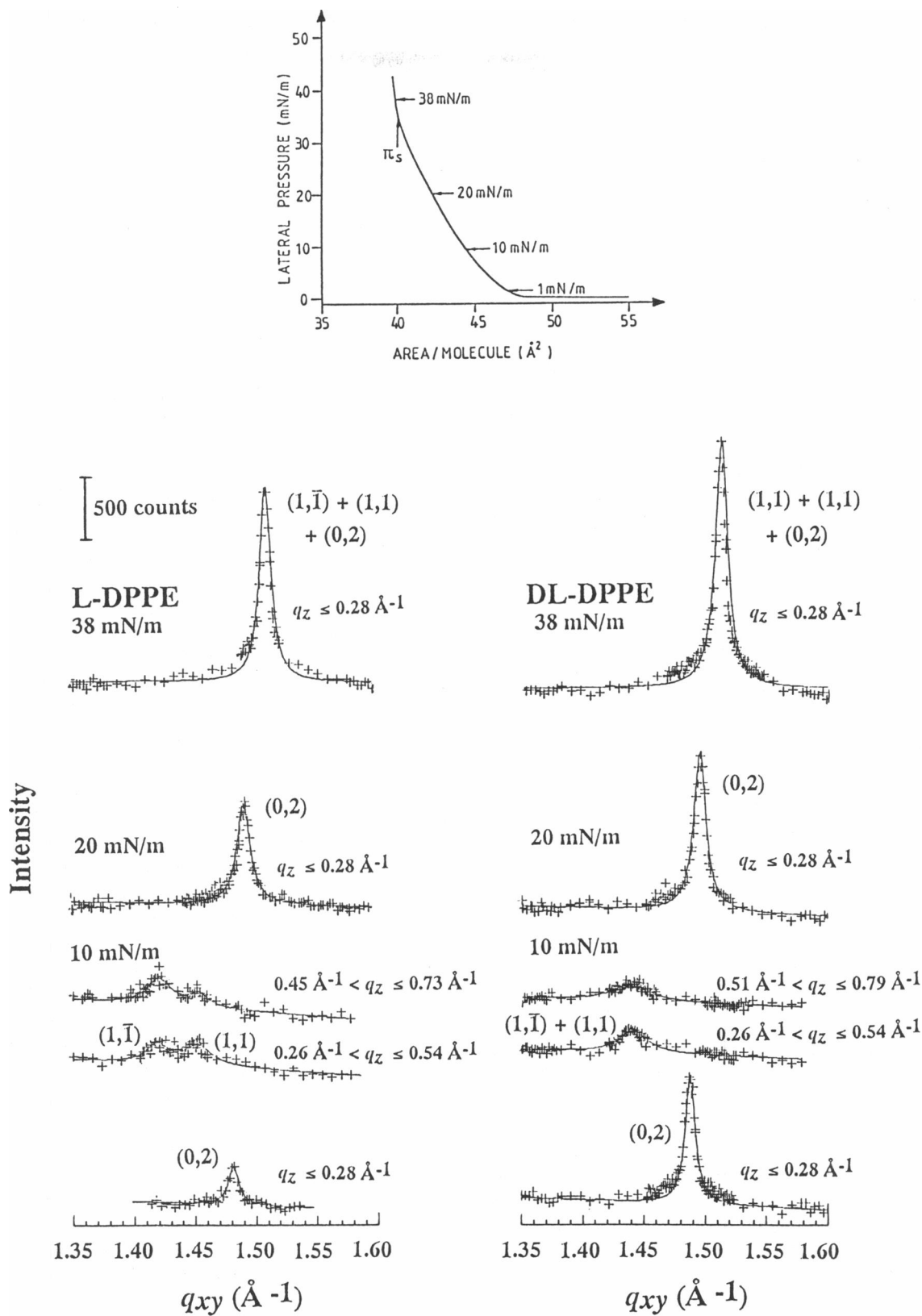


FIGURE 1 Scattering intensity as a function of in-plane wave-vector transfer  $q_{xy}$  for different  $q_z$  intervals (indicated) for monolayers of L-DPPE (left) and DL-DPPE (right) at different surface pressures. The inset gives the surface pressure/area isotherm ( $T = 20^\circ\text{C}$ ,  $\text{pH} \approx 6$ ) which is identical for both compounds, with arrows indicating the positions at which diffraction data have been taken. The slope change at  $\pi_s$  is also marked.

behaviour of the peaks observed at higher  $q_z$  values: The mixture displays one peak that splits into two for the pure compound.

## ANALYSIS

The 2-D lattice of the monolayer can be extracted from the  $q_{xy}$  positions of the different GID peaks. The lattice spacings  $d_{hk}$  are given by:

$$d_{hk} = \frac{2\pi}{q_{hk}^m}, \quad (1)$$

where  $q_{hk}^m$  corresponds to the maximum intensity of the Bragg peaks. The two peaks for the racemate describe a pure rectangular cell; the three peaks for L-DPPE describe an oblique cell. The latter may be considered pseudo rectangular, because the angle  $\gamma$  between the  $a$  and  $b$  axes is very close to  $90^\circ$  (see Fig. 4 and Table 1). The lattice spacings, the derived unit cell dimensions and area per molecule  $A_{xy} = ab \sin \gamma$ , of L-DPPE and DL-DPPE at different surface pressures are listed in Table 1.

If the aliphatic tails are uniformly tilted by an angle  $t$  with respect to the surface normal and with an angle  $\Psi_{hk}$  between the molecular axis projected on the basal plane and the lattice plane, the position of the maximum intensity along a Bragg rod profile  $q_{z,hk}^m$  is given by the equation (11):

$$\frac{q_{z,hk}^m}{q_{hk}^m} = \tan t \sin \Psi_{hk}. \quad (2)$$

The chains are tilted perpendicular to the  $b$ -axis because the  $d_{02}$  peak is maximal for  $q_z = 0$ . The vertical component  $q_{z,hk}^m$  is estimated from the data given in the figures, resulting in an error limit of  $\pm 3^\circ$  for the tilt angle.

The cross section per chain  $A_0$  can be derived from

$$A_0 = A_{xy} \cos t. \quad (3)$$

The values of  $A_0$  and  $t$  are included in Table 1.

The following structural data may be deduced from Table 1:

(a) The cross section  $A_0$  per molecule for both compounds is nearly independent of pressure and as expected for alkanes in their free rotator phases (12). There are no differences for the chiral resolved compound and racemate.

(b) The molecular surface area  $A_{xy}$  is slightly (but systematically) smaller for the racemate and, as expected, decreases with increasing pressure. This correlates with the change in tilt angle according to Eq. 3 for  $A_0 = \text{constant}$ .

(c) The molecules are tilted in a direction towards nearest neighbours.

(d) Comparing L-DPPE and DL-DPPE at the same pressures below  $\pi_s$  the pure compound has a slightly larger spacing  $d_{02}$ , i.e., normal to the tilt direction. These differences, which are also encountered for the other directions are within the error limits, taking into account that the pressures to be compared may differ by 2 mN/m. Yet, the fact that in all cases the racemate was systematically found to be more compact, suggests that this effect is real.

## DISCUSSION

Fig. 4 depicts the lattices given by the projection of the tails on the surface for the two systems at a pressure below  $\pi_s$  together with arrows describing the projection of the tilt azimuth. At the present stage we have no conclusive results on the conformation of the head group linking two chains. We hope to achieve this by a more accurate measurement of Bragg rod scans in conjunction with computer simulations. Still there is now enough evidence supporting head group ordering:

(a) The rectangular cell deduced from the two reflections of DL-DPPE indicates the presence of glide symmetry. The close fit of the GID data of the L-DPPE to that of the racemate DL-DPPE suggests a similar packing, but the molecules of the chiral resolved compound cannot be related by glide plane, leading to a symmetry break. These arguments are only valid if the head groups tend to be ordered.

(b) In order to glean more information on the head group packing we performed crystallization experiments of PEA under monolayers of L-DPPE and DL-DPPE. We took advantage of the observation that the 3-D crystals of PEA (2, 3) and the 3-D phospholipid DL-DLPE (1) form very similar hydrogen-bonded layers in which the molecules are related by translation and glide symmetry. Thus, the phospholipid monolayers can be used as a template for the crystallization of PEA (13). We observed that PEA crystals are grown under the chiral resolved L-DPPE monolayer, but not under the racemate DL-DPPE, i.e., that the head group must be ordered. However, we cannot state at this stage whether the absence of crystallization of PEA under the racemate monolayer is due to a disordered head group arrangement or a mismatch.

(c) It is gratifying that orientational order of the head group of L-DMPE has been observed by atomic force microscopy with molecular resolution (14) though one might argue that this technique fixes structures before observing them.

Previous indications on head group order were derived from measurements and analysis of pressure/area isotherm and fluorescence microscopy data with DMPE (4). These indicated ordering of tails coupled with those

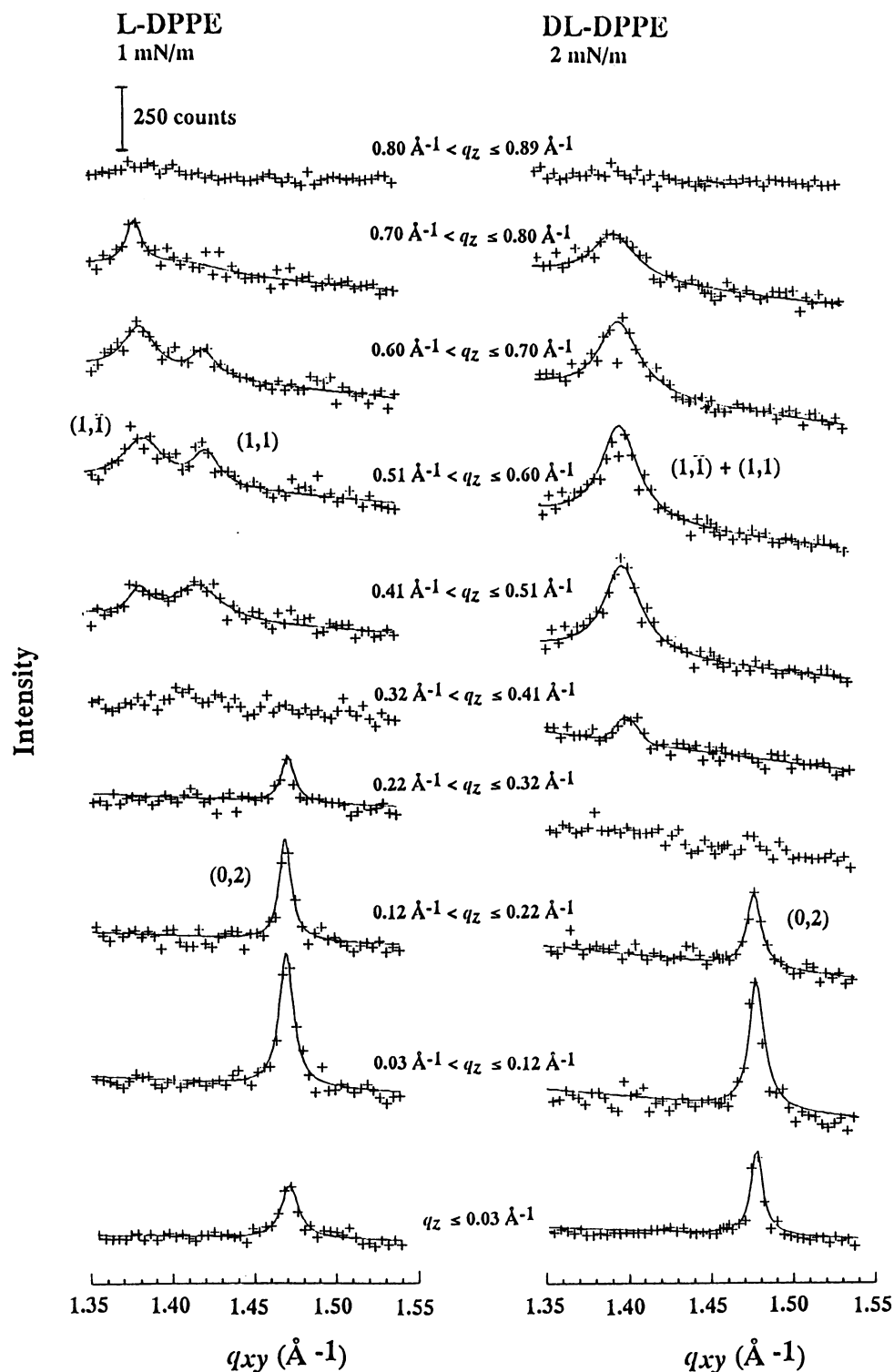


FIGURE 2  $q_z$  resolved in-plane diffraction scans for L-DPPE (left) and DL-DPPE (right) monolayers at very low surface pressures (indicated).

of the head groups at temperatures above 21°C and decoupled at lower temperatures. In addition, surface potential data with DMPE suggested head group rearrangements on compressing the monolayer (Miller et al.,

1987). It may transpire that these data have to be reinterpreted, because changes in the surface potential may also be due to changes in the water structure shielding polar groups.

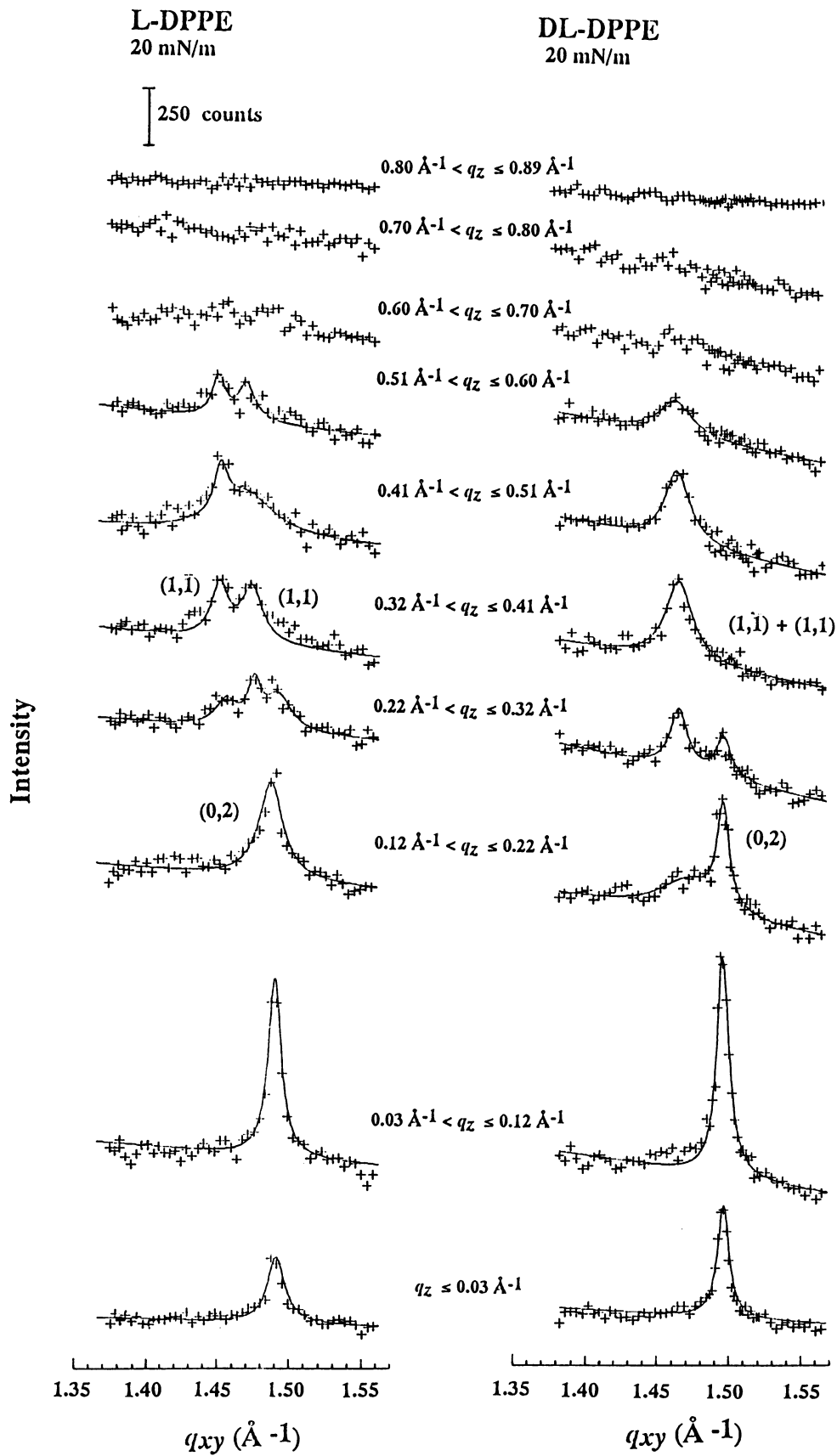


FIGURE 3  $q_z$  resolved in-plane diffraction scans for L-DPPE (left) and DL-DPPE (right) monolayers at 20 mN/m.

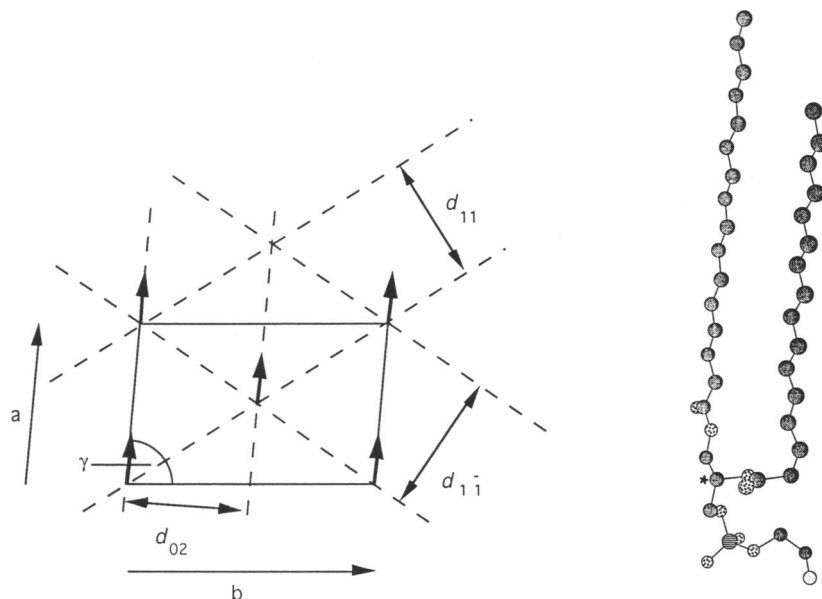


FIGURE 4 (Left) Two-dimensional unit cell containing one molecule, i.e., containing one chain in the corner and one in the center. The axes  $a$ ,  $b$ , the angle  $\gamma$  and the  $d$ -spacings are indicated. The arrows designate the orientation of the tilt azimuth which is almost parallel to the  $a$  axis. (Right) Molecular structure of DPPE marking by an asterisk the chiral center; (●) C-Atom, (○) O-Atom, (⊙) P-Atom, (⊗) N-Atom.

There is an essential difference in packing density for the monolayer compared to the three dimensional crystal structures. In the chiral phosphorylcholine L-DMPC the hydrocarbon cross section is  $19.0 \text{ \AA}^2$  (15) and in the phosphoryl-ethanolamine DL-DLPE  $19.3 \text{ \AA}^2$  (1), whereas in the DPPE monolayer it is 3-4% higher ( $19.8 \text{ \AA}^2$ ). In addition the three dimensional crystals contain at most two water molecules.

It is also interesting to compare the structures observed with data obtained previously for arachidic acid (16). There a chain tilt to a nearest neighbour chain was observed which is continuously reduced on compression, and the cross section per chain amounts to  $19.8 \text{ \AA}^2$ . This is exactly what is observed for the racemate in this

work, but there is a quantitative difference in the lattice anisotropy which becomes obvious from a comparison of  $d_{02}$  and  $d_{11}$  spacings at 10 mN/m. For the single chain compound  $d_{02} = 4.26 \text{ \AA}$  and  $d_{11} = 4.31 \text{ \AA}$  were observed whereas the data in Fig. 1 reveal  $d_{02} = 4.23 \text{ \AA}$  and  $d_{11} = 4.37 \text{ \AA}$ . Hence the coupling of two chains by the head group causes a larger expansion of the lattice into the direction of chain tilt.

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TABLE 1 Lattice spacings, unit cell dimension and projected molecular area  $A_{xy}$  as derived from in-plane diffraction data at different surface pressures for the chirally resolved compound and for the racemate. Tilt angle  $t$  and molecular cross section  $A_o$  were derived as described in the text

Surface pressure $\pi$	Sample	Lattice spacings			Unit cell dimensions			Molecular area $A_{xy}$	Tilt angle	Cross section $A_o$
		$d_{11}$	$d_{11}$	$d_{02}$	$a$	$b$	$\gamma$			
<i>mN/m</i>			$\text{\AA}$		$\text{\AA}$	$\text{\AA}$	$^\circ$	$\text{\AA}^2$	$^\circ$	$\text{\AA}^2$
1	L	4.53	4.42	4.27	5.25	8.54	91.6	44.8	29	39.3
	DL	4.47	4.47	4.25	5.26	8.50	90.0	44.6	27	39.9
10	L	4.42	4.32	4.27	5.10	8.48	91.5	43.3	24	39.6
	DL	4.37	4.37	4.23	5.10	8.46	90.0	43.2	22	40.0
20	L	4.33	4.27	4.21	5.00	8.42	90.9	42.1	18	40.1
	DL	4.28	4.28	4.20	4.97	8.40	90.0	41.8	18	39.8
38	L	4.15	4.15	4.15	4.79	8.30	90.0	39.7	0	39.7
	DL	4.14	4.14	4.14	4.78	8.28	90.0	39.6	0	39.6

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