Chiral Discrimination in a Monolayer of a Triple-Chain Phosphatidylcholine

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ABSTRACT Monolayers of an enantiomeric and a racemic triple-chain phosphatidylcholine (PC) at the air/water interface are studied by film balance measurements and x-ray diffraction. Although the area per three tails exceeds that per head, we observe tail ordering dependent on headgroup chirality and chain tilt. This indicates lateral headgroup interactions. The influence of the chiral carbon is suppressed at higher lateral pressures, and a centered-rectangular unit cell with tails tilted into the nearest neighbor (NN) direction is observed for both the enantiomer and the racemate. The distortion of the lattice changes at medium pressures from NN to NNN (next-nearest neighbor direction) with decreasing temperature. The phase behavior of the racemate at 15°C is compared with that of a triple-chain PC with a branched chain of reduced length. Whereas the PC with the longer branched chain exhibits only a NN tilted phase at all pressures, the PC with the shorter branched chain has a rich polymorphism (NNN–NN–upright hexagonal packing) under increased lateral pressure.

INTRODUCTION

Although the richness of mesophases of amphiphiles at the air/water interface has been sufficiently characterized (Kenn et al., 1991; Bibo et al., 1991), there remain many open questions regarding the underlying interactions. One of these questions concerns the interplay between forces involving the headgroup and the tail regions, which is important in understanding phospholipid monolayers. These competitive interactions are manifested in a variety of known facts.

For monolayers of 1,2-dipalmitoyl-phosphatidylcholine (DPPC) it is known that because of the large head the tails cannot align vertically, even at the highest lateral pressure (Brezesinski et al., 1995b).

For enantiomeric DPPC, chiral domains of the ordered phase have been observed (Weiss and McConnell, 1984). X-ray diffraction has also revealed a chiral structure of the aliphatic tails. However, this was observed also for the racemate (Brezesinski et al., 1995b), although there domains do not display chirality (Moy et al., 1988).

The above finding contrasts with that for 1,2-dipalmitoyl-phosphatidylethanolamine (DPPE), where a chiral lattice was observed for the enantiomer, not for the racemate (Böhm, 1993; Böhm et al., 1993).

Chiral interactions are by themselves an interesting object in physics and biology (Jaques et al., 1981). Chiral discrimination was investigated theoretically for chiral molecules forming an insoluble monolayer at the air/water interface (Andelman, 1989, 1990; Andelman and Orland, 1993). Fluorescence microscopy reveals distinct differences in the condensed phase domain shapes between the enantiomeric and racemic monolayers (Weiss and McConnell, 1984; Moy et al., 1988; Stine et al., 1993; Parazak et al., 1994). Recently a spontaneous chiral segregation has been found in Langmuir films of a racemic mixture of myristoyl-alanine by epifluorescence microscopy and x-ray diffraction (Nassoy et al., 1995). However, the occurrence of an oblique lattice for racemates could also be due to spontaneous symmetry breaking, which would not require any phase separation (Viswanathan et al., 1994). This was observed even for monolayers of nonchiral molecules. Yet here we are predominantly interested in the fact that slight chemical changes in both the hydrophobic and hydrophilic regions as well as at the hydrophobic/hydrophilic interface (chirality of the glycerol backbone) can have a strong influence on tail ordering. To gain further information on this we studied the enantiomer and the racemate of a phosphatidylcholine with three aliphatic tails. Moreover, hydrated phosphatidylcholines containing 2-alkyl substituted fatty acyl chains are interesting systems because they can form nonlamellar phases in bulk at physiologically relevant temperatures (Nuhn et al., 1986; Lewis et al., 1994). In monolayers at the air/water interface we expect the tails of the triple-chain lipid to determine the packing. We have characterized the phase behavior of the enantiomer and the racemate using pressure-area isotherms as well as x-ray diffraction. By grazing incidence x-ray diffraction one can study the microscopic (Å-scale) monolayer structure in considerable detail (Als-Nielsen and Kjaer, 1989; Als-Nielsen and Möhwald, 1991). To our surprise, chirality is still reflected in the tail ordering at lower lateral pressures, and a strong headgroup repulsion is revealed.

MATERIALS AND METHODS

Both the racemic mixture and one of the enantiomers have been prepared using well-known procedures described in the literature (Paltauf and Hermetter, 1994). The synthesis of the racemate starts from commercially

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available racemic 1,2-isopropyliden-glycerol, which was converted to 1-*O*-hexadecylglycerol (1). Tritylation of 1 followed by benzylation, removal of the trityl residue, phosphorylation, and conversion to the phosphatidylcholine resulted in racemic 1-*O*-hexadecyl-2-benzyl-glycero-3-phosphocholine. After catalytic removal of the benzylether, acylation with the 2-hexadecylstearic acid anhydride in the presence of catalytic amounts of 4,4′-dimethylaminopyridine led to the racemic triple-chain 1-*O*-hexadecyl-2-(2′-hexadecylstearoyl)-glycero-3-phosphocholine (rac. ^{1}H-2-($2C_{16}$-18:0)-PC). Starting the synthesis from 1,2-isopropylidene-*sn*-glycerol (Aldrich, Germany), which was converted to 3-*O*-hexadecyl-*sn*-glycerol, the corresponding enantiomeric 3-*O*-hexadecyl-2-(2′-hexadecylstearoyl)-*sn*-glycero-1-phosphocholine (3H-2-($2C_{16}$-18:0)-*sn*-1-PC) could be obtained by using the same procedures as described previously for the racemates (Rattay et al., 1995). All final compounds have been purified by column chromatography. The characterization has been performed by analytical high-performance liquid chromatography (HPLC) and electrospray mass spectroscopy.

Selected analytical data

(^{1}H-2-($2C_{16}$-18:0)-PC): rac. 1-*O*-hexadecyl-2-(2′-hexadecylstearoyl)-glycero-3-phosphocholine, $C_{58}H_{118}N_1O_7P_1$; M = 972.5 gmol^{-1}; $[M+H]^+$ = 973.0; $[M+Na]^+$ = 995.1; HPLC: t_R = 9.30 min; micro analysis (%) P (calc/obt.): 3.19/3.05.

(3H-2-($2C_{16}$-18:0)-*sn*-1-PC): 3-*O*-hexadecyl-2-(2′-hexadecylstearoyl)-glycero-sn-1-phospho-choline, $C_{58}H_{118}N_1O_7P_1$; M = 972.5 gmol^{-1}; $[M+H]^+$ = 973.1; $[M+Na]^+$ = 995.2; HPLC: t_R = 9.34 min; micro analysis (%) P (calc/obt.): 3.19/3.09; $\alpha_D^{[20]} = -9.42°$ (0.01 mol/L, $CHCl_3$).

The HPLC measurements of the lipids prepared were performed with a JASCO chromatograph fitted with a 250 × 4.6 mm Kromasil Si 100 column (100–5 μm) (JASCO) equipped with an evaporative light-scattering detector (ELSD IIA). Chloroform/methanol/water (60/40/4, v/v/v) was used as the mobile phase. The flow rate was 0.5 ml/min for 10 min and then 1 ml/min until the end of the analysis. The column temperature was maintained at 20°C. The lipids were diluted in sufficient quantities of standard mixtures of chloroform/methanol (80/20, v/v), and 20 μl of the solution was injected. Chromatograms and integrated data were recorded with a JASCO Chromatopac.

Calorimetric studies were performed with the DSC-2 apparatus (Perkin-Elmer) using bulk lipid in a water (50 wt%) saturated state (Nuhn et al., 1986).

Pressure/area isotherms were recorded by a home-built film balance with a continuous Wilhelmy-type pressure-measuring system (Dietrich et al., 1991) in a temperature range between 5°C and 40°C. The compression speed was 3.1×10^{-2} nm^2/(min·molecule). The subphase was ultrapure water with a specific resistance above 18 MΩcm, and was purified using a Millipore desktop unit.

Synchrotron grazing incidence x-ray diffraction (GID) experiments were performed at 5°C, 15°C, and 25°C using the liquid-surface diffractometer at the undulator beamline BW1 (Kjaer et al., 1992; Als-Nielsen et al., 1994) at HASYLAB, DESY (Hamburg, Germany). The x-ray beam was made monochromatic and kept below the critical angle for total external reflection. This limits the penetration depth of the beam to that of the evanescent wave (~100 Å). A linear position-sensitive detector (OED-100-M; Braun, Garching, Germany) measured the diffraction intensity as a function of the vertical scattering angle α_f. The horizontal scattering angle $2\theta_{xy}$ was varied by rotating the PSD together with a horizontally collimating Soller slit, mounted in front of the PSD, around a vertical axis through the center of the sample. The lattice parameters, tilt angle, and tilt direction can be calculated from the horizontal ($Q_{xy} \cong (4\pi/\lambda)\sin(2\theta_{xy}/2)$) and vertical ($Q_z \cong (2\pi/\lambda)\sin\alpha_f$) components of the scattering vector **Q** (Als-Nielsen and Kjaer, 1989; Als-Nielsen and Möhwald, 1991; Jacquemain et al., 1992; Als-Nielsen et al., 1994; Kjaer, 1994; Struth et al., 1994; Brezesinski et al., 1995b).

RESULTS

Pressure/area isotherms

Both the pure enantiomeric 3H-2-($2C_{16}$-18:0)-*sn*-1-PC and the corresponding racemate ^{1}H-2-($2C_{16}$-18:0)-PC form stable monolayers at the air-water interface. Fig. 1 shows the surface pressure as a function of molecular area at three different temperatures. At lower temperatures the isotherms are fully condensed. Above a certain temperature T_0 the first-order transition from a liquid-expanded state to a condensed phase can be observed. The corresponding transition pressure π_c does not depend on chirality. Above 26°C π_c is a linear function of temperature, and the slope gradually decreases on going to lower temperatures. The extrapolation of the transition pressure π_c toward zero yields the T_0 values, which amount to (23 ± 0.5)°C. The transition enthalpy ΔH can be calculated, using a Clausius-Clapeyron equation, from the area change ΔA involved in this transition (Albrecht et al., 1978; Dietrich et al., 1991). At 26°C ΔH is about 88 kJ/mol and decreases with increasing temperature. ΔH versus temperature (see Fig. 2) is characterized by two linear ranges with a drastic change in slope. An extrapolation of ΔA or ΔH toward zero yields a critical temperature T_c = 72 (± 3)°C. The isotherms measured for the racemate and the enantiomer exhibit one important difference: there is a very small additional change in area or a pronounced change in slope (indicated by a dotted arrow) at pressures π_s (above π_c) for the enantiomer but not for the racemate. This could be an indication of a transition between two condensed states in the film of the enantiomer. Inspecting the isotherms in Fig. 1 one observes a π_s value of about 6 mN/m at 5°C and about 15 mN/m at 25°C. In the isotherm at 15°C this transition is not detectable. From these data we could conclude that π_s is slightly increasing with increasing temperature.

X-ray diffraction

Fig. 3 depicts the diffracted intensity as a function of the in-plane scattering vector component Q_{xy} of the enantiomeric 3H-2-($2C_{16}$-18:0)-*sn*-1-PC at 25°C and 7 mN/m. The diffracted intensity is plotted in Q_z intervals (0.08 Å^{-1}) with increasing Q_z from bottom to top. The width of the maximum for $Q_z > 0$ Å^{-1} indicates that two peaks contribute. The fitted maximum positions of these diffraction peaks are marked by bars. There is a large overlap between the peaks with higher Q_z. Fig. 4 shows the diffraction intensity along the plane normal (z) (Bragg rod), integrated over small Q_{xy} intervals (indicated), around the maximum positions of the three peaks observed for 7 mN/m. In this case we can clearly distinguish three different Bragg rods and therefore prove the existence of three diffraction peaks. Between 13 mN/m and 30 mN/m the two peaks at higher Q_z merge, indicating a transition from an oblique to a centered-rectangular unit cell. Further increase of the lateral pressure moves

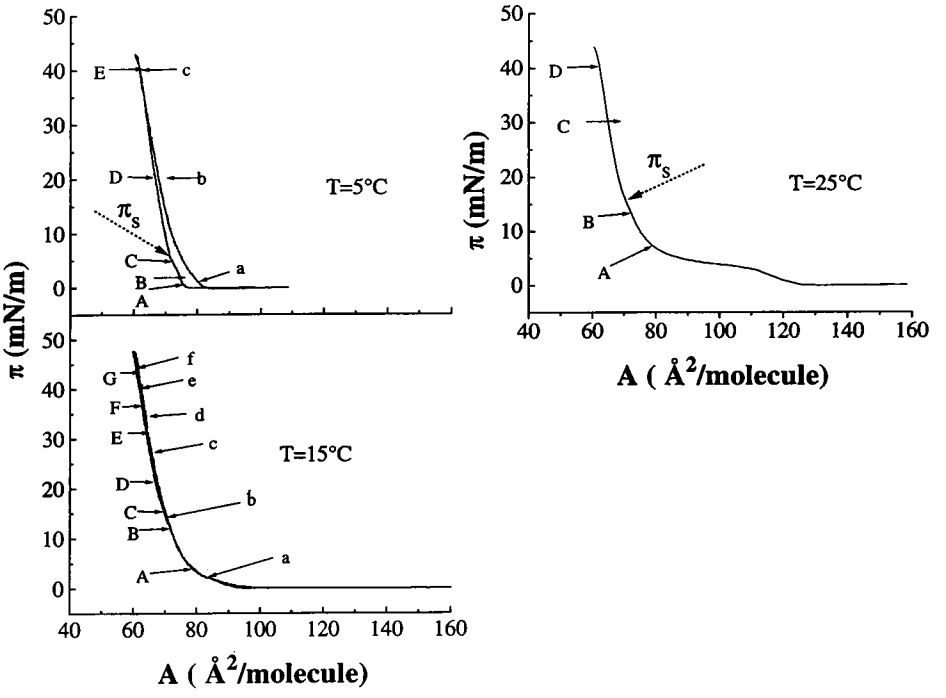

FIGURE 1 Surface pressure (π)–molecular area (A) isotherms for monolayers of the enantiomeric 3-*O*-hexadecyl-2-(2′-hexadecylstearoyl)-glycero-sn-1-phosphocholine (*capital letters*) and the racemic 1-*O*-hexadecyl-2-(2′-hexadecylstearoyl)-glycero-3-phosphocholine (*small letters*) on aqueous substrate at different temperatures. The points *A–G* (*a–f*) indicate the surface pressures at which diffraction data have been taken. The pressure π_s shows a transition between two condensed states of the enantiomeric monolayer.

the twofold degenerate peak to lower Q_z and higher Q_{xy} values, and at 40 mN/m as well as at 45 mN/m the same Q_{xy} value is observed for the two peaks. At 15°C the contour plots of the corrected x-ray intensities as a function of in-plane scattering vector component Q_{xy} and out-of-plane scattering vector component Q_z show at the lowest surface pressure of 4 mN/m that the enantiomer displays three distinct diffraction peaks, indicating again molecular tilt in a nonsymmetry direction and hence the absence of mirror symmetry. This is concluded from the asymmetry of the contour at higher Q_z, although the two peaks are barely resolved (Fig. 5). With increasing pressure these two peaks merge again into one, and the third peak occurs at $Q_z = 0$ Å^{-1}. Further increase of the lateral pressure shifts the twofold degenerate peak to lower Q_z values and to Q_{xy} values closer to those of the nondegenerate peak. At a certain pressure the two peaks exhibit the same Q_{xy} value. At still higher pressures the Q_{xy} positions of the degenerate and the nondegenerate peaks are changed. At 5°C the transition from the oblique to the rectangular unit cell occurs between 5 mN/m and 20 mN/m. In contrast to the measurement at higher temper-

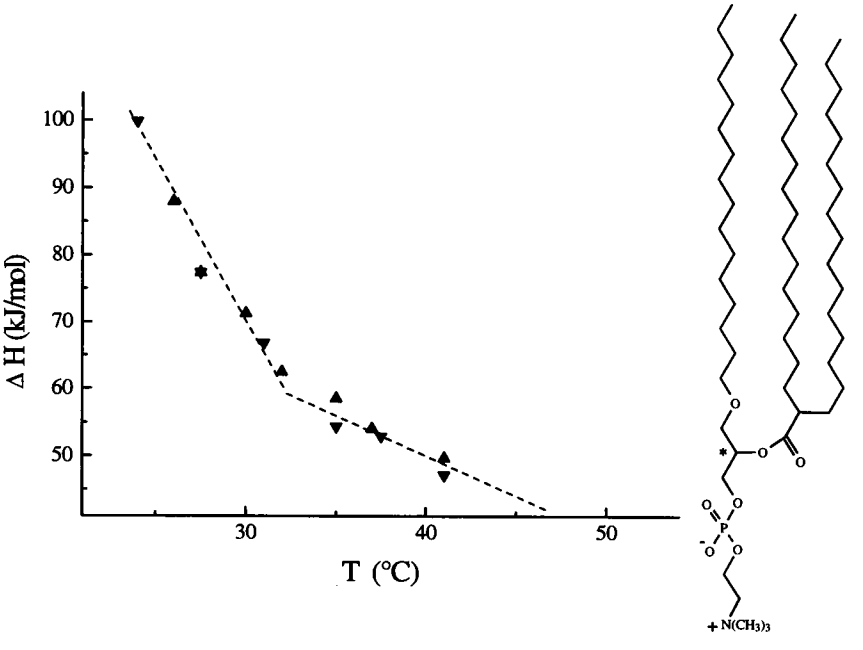

FIGURE 2 Enthalpy ΔH of the transition from a liquid expanded to a condensed phase as a function of temperature for monolayers of the racemic (▲) and the enantiomeric (▼) triple-chain phospholipid (*left*). (*Right*) Chemical structure of 3-O-hexadecyl-2-(2-hexadecylstearoyl)-glycero-sn-1-phosphocholine. The * marks the asymmetric carbon atom (chiral center).

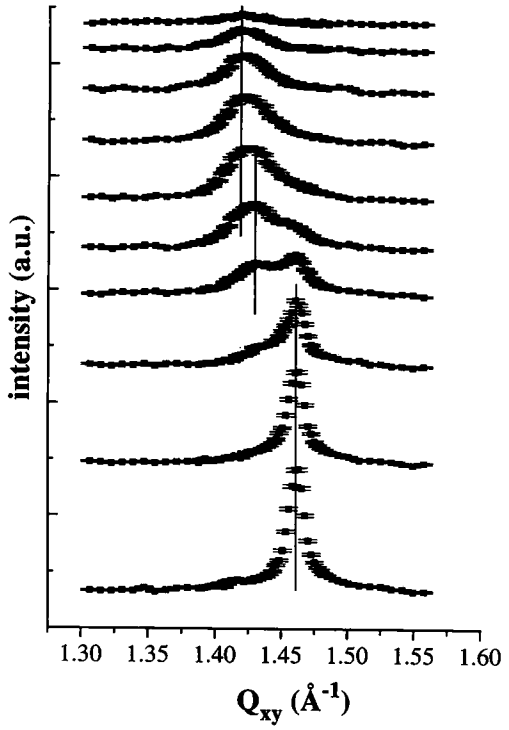

FIGURE 3 Scattering intensity as a function of in-plane scattering vector component Q_{xy} for different Q_z intervals (0.08 Å^{-1} with increasing Q_z from bottom to top) for monolayers of the enantiomeric 3H-2-($2C_{16}$-18:0)-*sn*-1-PC at 25°C and 7 mN/m.

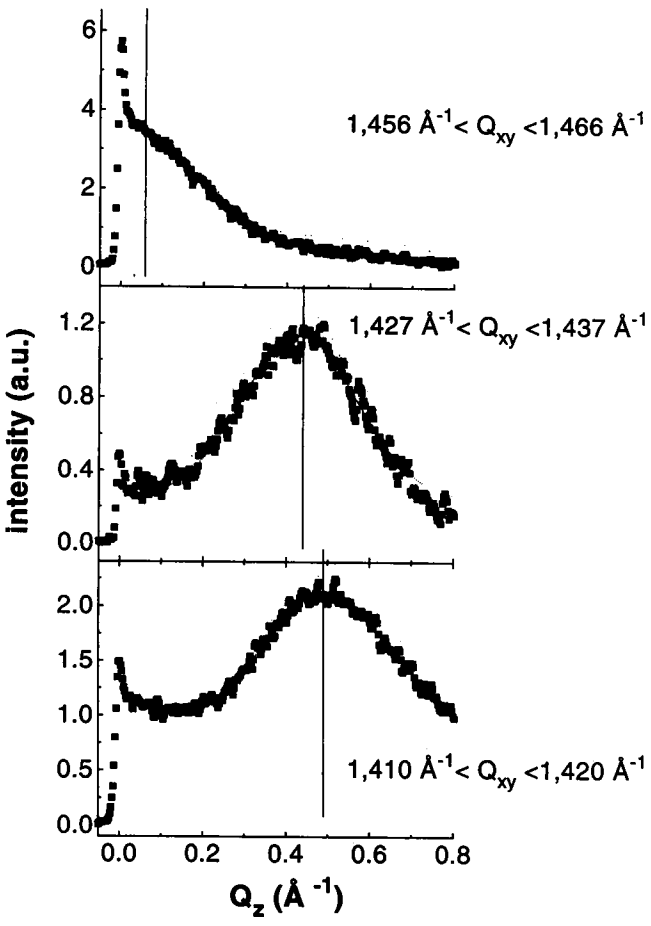

FIGURE 4 Diffraction intensity as a function of out-of-plane scattering vector component Q_z integrated over small Q_{xy} intervals (indicated) for monolayers of the enantiomeric 3H-2-($2C_{16}$-18:0)-*sn*-1-PC at 25°C and 7 mN/m.

atures, the twofold degenerate peak occurs at higher Q_{xy} values compared to those of the nondegenerate peak (Fig. 5).

At all temperatures and pressures investigated the racemate exhibits only two diffraction peaks. At 5°C the twofold degenerate peak occurs at higher Q_{xy} values than the nondegenerate peak. A reverse situation is observed at 15°C, and at higher pressures the two peaks exhibit the same Q_{xy} value.

The lattice parameters deduced from the diffraction data are summarized in Table 1. One deduces from Table 1 that the cross sections A_o per aliphatic tail are as expected for alkane rotator phases. A_o is reduced with decreasing temperature (from 20.6 Å^2 at 25°C to 19.8 Å^2 at 5°C). However, within experimental error there are no differences between enantiomer and racemate. Also as expected, the tilt

TABLE 1 Racemic 1-hexadecyl-2-(2'-hexadecylstearoyl)-glycero-3-phosphocholine at T = 15°C and 5°C

π [mN/m]	a [Å^{-1}]	b [Å^{-1}]	γ [°]	t [°]	ξ	A_{XY} [Å^2]	A_O [Å^2]
				T = 15°C			
2	5.12	5.03	120.6	23.4	0.0224	22.2	20.4
14	5.05	4.98	120.4	20.5	0.0184	21.7	20.3
27	4.98	4.96	120.1	18.8	0.0046	21.4	20.2
34	4.94	4.94	120.0	17.3	0	21.1	20.2
40	4.89	4.89	120.0	12.8	0	20.7	20.2
44	4.87	4.87	120.0	11.4	0	20.6	20.2
48	4.84	4.84	120.0	7.8	0	20.4	20.2
				T = 5°C			
1	4.97	5.06	119.4	25.7	0.0249	21.9	19.8
20	4.81	5.03	118.6	22.0	0.0578	21.2	19.7
40	4.67	4.96	118.1	16.7	0.0770	20.5	19.6

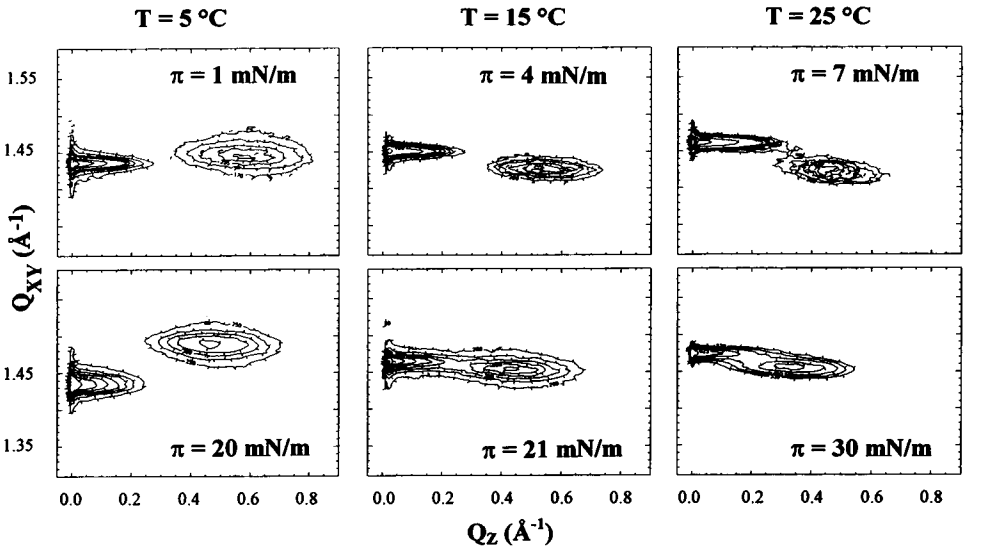

FIGURE 5 Contour plots of the corrected x-ray intensities as a function of the in-plane component Q_{xy} and the out-of-plane component Q_z of the scattering vector Q of the enantiomeric 3H-2-(2C_{16}-18:0)-*sn*-1-PC at different temperatures and lateral pressures (indicated).

angle is reduced with increasing pressure. This reduction is nearly linear, as displayed in Fig. 6.

DISCUSSION

The present experiments have demonstrated that the asymmetric carbon of the glycerol backbone has an influence on the monolayer structure that is detected at low surface pressures. Fig. 5 compares the contour plots of the enantiomer at lower and higher surface pressures and at three different temperatures. The two peaks at high Q_z are very close, and the maximum of the third peak is close to Q_z = 0 Å^{-1}. This indicates that the deviation from the rectangular unit cell due to the chiral carbon of the glycerol is not very pronounced. The increase in the lateral pressure suppresses this influence completely, and a centered-rectangular unit cell is found at higher pressures. Whereas the enantiomer forms a chiral (oblique) lattice structure, the racemate exhibits only two diffraction peaks leading to a centered-rectangular unit cell. In the case of the double-chain phosphatidylcholine DPPC an oblique phase was observed for both the enantiomer and the racemate (Brezesinski et al., 1995b). A possible explanation of this observation was the orientational ordering of the glycerol backbones linking the two hydrocarbon chains at an oblique angle. This could be established irrespective of chirality. On the other hand, in the case of the 1,2-dipalmitoyl-phosphatidylethanolamine (DPPE) only the enantiomer exhibits a chiral structure (Böhm et al., 1993). Therefore, for the enantiomer a hydrogen bonding network between neighboring headgroups parallel to the *b* axis of the unit cell was assumed, whereas the racemate forms a two-dimensional network of hydrogen bonds (Böhm, 1993).

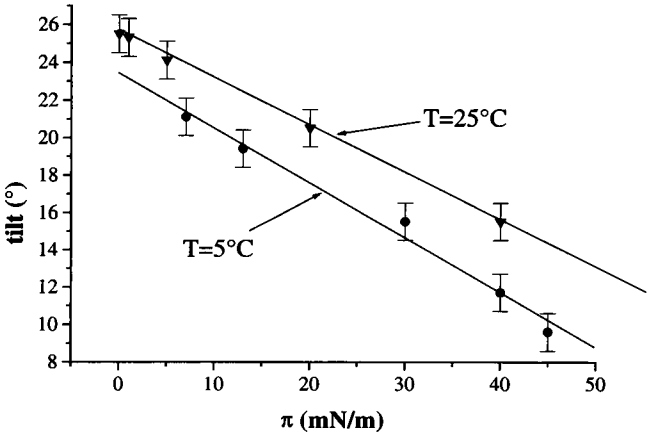

FIGURE 6 Tilt angle t as a function of lateral pressure π for the monolayer of the enantiomeric 3H-2-(2C_{16}-18:0)-*sn*-1-PC at two different temperatures.

In the present case the PC headgroup is bulky, but the three chains require more space and therefore determine the area at dense packing. The observed influence of the chiral carbon atom indicates that it is not sufficient to consider area requirements; the extension of the headgroup in specific directions must also be considered. For example, inspection of the molecular structure reveals that the distance between the chiral carbon and the trimethylammonium group is about 10 Å, i.e., larger than two lattice spacings. Therefore, if this group is oriented parallel to the surface, a local interaction with the adjacent headgroup is still possible. Such an interaction is also suggested by the fact that even at the highest pressure the chains are tilted. This contrasts with the finding that the aliphatic tails at high pressure form a hexagonal lattice with vertical alignment if the tail interaction determines the structure. Hence we conclude that the headgroups are not decoupled in this system, because they align predominantly parallel to the surface.

The aqueous (50 wt% water) bulk systems also show only a small influence of chirality on the parameters of the transition between the gel and liquid-crystalline states. The transition temperatures T_m amount to 53.9°C for the race-

mate and to 52.4°C for the enantiomer. The corresponding transition enthalpies are 49.3 kJ/mol and 47.7 kJ/mol, respectively. The hydration value at 0°C, determined from the ice-peak evaluation, amounts to 24 mol water/mol lipid and is surprisingly large. For both the enantiomer and the racemate the critical temperature T_c of the monolayer is more than 20°C higher than the main transition temperatures T_m of the bulk lipid dispersions. The same behavior was already found for the positional isomers 1-*O*-hexadecyl-2-(2-tetradecylpalmitoyl)-glycero-3-phosphocholine (^{1}H-2-($2C_{14}$-16:0)-PC) and 1-(2-tetradecylpalmi-toyl)-2-*O*-hexadecyl-glycero-3-phosphocholine (Dietrich et al., 1991) and explained by a model on head/head interactions between opposing monolayers in a bilayer arrangement.

To compare the corresponding racemic triple-chain PCs it is useful to introduce a second-order parameter in addition to the tilt of the chains. This parameter is the unit cell distortion. Its magnitude ξ is defined as $\xi = (l_1^2 - l_2^2)/(l_1^2 + l_2^2)$, where l_1 and l_2 are, respectively, the major and minor axes of the ellipse passing through all six nearest neighbors of a hydrocarbon chain (chain lattice). The distortion azimuth ω is the azimuth of the major axis of the ellipse (Kaganer et al., 1993, 1995; Kaganer and Loginov, 1995; Scalas et al., 1995).

The ^{1}H-2-($2C_{14}$-16:0)-PC, with three chains of almost the same length, has been described previously (Dietrich et al., 1991, 1994; Brezesinski et al., 1994, 1995a). Fig. 7 compares the contour plots of the racemic ^{1}H-2-($2C_{16}$-18:0)-PC with those of the racemic ^{1}H-2-($2C_{14}$-16:0)-PC at 15°C. The difference in length of the branched chain obviously has an influence on the phase behavior. At all pressures investigated, ^{1}H-2-($2C_{16}$-18:0)-PC exhibits a centered-rectangular lattice with chains tilted in a symmetry direction toward nearest neighbors (NN). At lower surface pressures the lattice is distorted from hexagonal packing in the NN direction ($\mathbf{Q}_{xy}^n > \mathbf{Q}_{xy}^d$), where $\mathbf{Q}_{xy}^n$ is the maximum position of the nondegenerate peak and $\mathbf{Q}_{xy}^d$ is the maximum position of the twofold degenerate peak (Kaganer et al., 1995). With increasing pressure the distortion decreases, and at 40 mN/m the lattice is undistorted (see Fig. 7). We observe a hexagonal in-plane unit cell in spite of a large tilt angle (t = 13°). In contrast to this behavior, 1H-2-($2C_{14}$-16:0)-PC shows a very rich polymorphism. At lower surface pressures a centered-rectangular packing with chains tilted toward next-nearest neighbors (NNN) was found; both the nondegenerate and the twofold degenerate peaks are above the horizon, with $\mathbf{Q}_z^n = 2\mathbf{Q}_z^d$. The lattice distortion azimuth is also in the NNN direction. Between 20 mN/m and 30 mN/m both directions change to NN. This change is connected with a very small decrease in the tilt angle. At 40 mN/m only one diffraction peak at $Q_z = 0$ Å^{-1} has been found, indicating a hexagonal packing of upright chains. It is known that the influence of an increase in chain length on the phase behavior is comparable to that of a decrease in temperature (Kenn et al., 1991). Comparing the phase behavior of these two molecules, we could expect that the pressure of the transition NNN–NN decreases with decreasing temperature

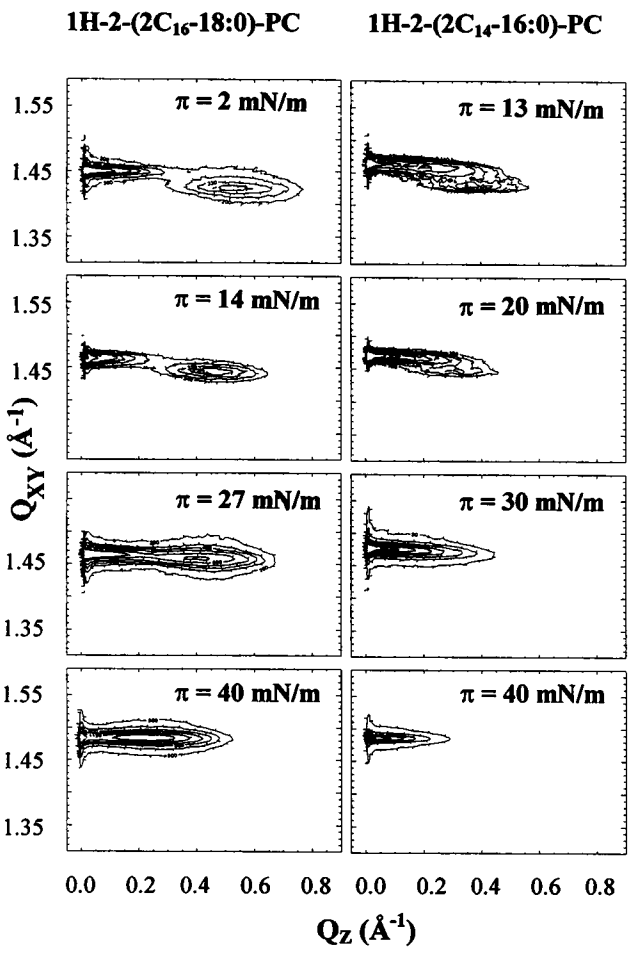

FIGURE 7 Contour plots of the corrected x-ray intensities as a function of the in-plane component Q_{xy} and the out-of-plane component Q_z of the scattering vector Q of the racemic 1-*O*-hexadecyl-2-(2-hexadecylstearoyl)-glycero-3-phosphocholine (^{1}H-2-($2C_{16}$-18:0)-PC) (*left*) and the racemic 1-*O*-hexadecyl-2-(2-tetradecylpalmitoyl)-glycero-3-phosphocholine (^{1}H-2-($2C_{14}$-16:0)-PC) (*right*) at 15°C and different lateral pressures (indicated).

(increasing chain length), whereas that of the transition NN–upright hexagonal packing increases. The temperature dependence of these transition pressures is so strong that for ^{1}H-2-($2C_{16}$-18:0)-PC only the NN phase can be observed in the pressure range from 0 to 50 mN/m. Comparing the structures of the enantiomeric ^{1}H-2-($2C_{16}$-18:0)-PC at medium pressure and different temperatures, one discovers within the NN tilted phase a change in distortion from NN (nearest neighbors) to NNN (next nearest neighbors) with decreasing temperature: the two diffraction peaks interchange continuously. Such a change in distortion was also observed in the L_{2h} phase of fatty acids (Kaganer et al., 1995).

It is interesting to note that the same phase sequence as for ^{1}H-2-($2C_{14}$-16:0)-PC has been observed for DPPC in contact with hexadecane (Brezesinski et al., 1995c). In the system DPPC/hexadecane, the incorporated hexadecane plays the role of branching. However, at 20°C the NNN–NN

transition occurs between 7 and 10 mN/m. This means that the covalent connection of the third chain with the main chain in the C2 position of the glycerol backbone decreases the pressure of the NNN–NN transition.

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REFERENCES

Albrecht, O., H. Gruler, and E. Sackmann. 1978. Polymorphism of phospholipid monolayers. *J. Phys. (Paris).* 39:301–313.

Als-Nielsen, J., and K. Kjaer. 1989. X-ray reflectivity and diffraction studies of liquid surfaces and surfactant monolayers. *In* Phase Transitions in Soft Condensed Matter. T. Riste and D. Sherrington, editors. Plenum Press, New York. 113–137.

Als-Nielsen, J., D. Jaquemain, K. Kjaer, M. Lahav, F. Leveiller, and L. Leiserowitz. 1994. Principles and applications of grazing incidence x-ray and neutron scattering from ordered molecular monolayers at the air-water interface. *Phys. Rep.* 246:251–321.

Als-Nielsen, J., and H. Möhwald. 1991. Synchrotron x-ray scattering studies of Langmuir films. *In* Handbook on Synchrotron Radiation, Vol. 4. S. Ebashi, M. Koch, and E. Rubinstein, editors. Elsevier/North Holland, Amsterdam. 1–53.

Andelman, D. 1989. Chiral discrimination and phase transitions in Langmuir monolayers. *J. Am. Chem. Soc.* 111:6536–6544.

Andelman, D. 1990. On the theory of tripod amphiphiles. Chiral discrimination and phase transitions in Langmuir monolayers. *Phys. A.* 168: 172–178.

Andelman, D., and H. Orland. 1993. Chiral discrimination in solutions and in Langmuir monolayers. *J. Am. Chem. Soc.* 115:12322–12329.

Bibo, A. M., C. M. Knobler, and I. R. Peterson. 1991. A monolayer phase miscibility comparison of long-chain fatty acids and their ethyl esters. *J. Phys. Chem.* 95:5591–5599.

Böhm, C. 1993. Ph.D. thesis. Universität Mainz.

Böhm, C., H. Möhwald, L. Leiserowitz, J. Als-Nielsen, and K. Kjaer. 1993. Influence of chirality on the structure of phospholipid monolayers. *Biophys. J* 64:553–559.

Brezesinski, G., C. Böhm, A. Dietrich, and H. Möhwald. 1994. Condensed phases in monolayers of a triple-chain lecithin on water. *Phys. B.* 198:146–149.

Brezesinski, G., A. Dietrich, B. Dobner, and H. Möhwald. 1995a. Morphology and structures in double-, triple- and quadruple-chain phospholipid monolayers at the air/water interface. *Prog. Colloid Polym. Sci.* 98:255–262.

Brezesinski, G., A. Dietrich, B. Struth, C. Böhm, W. G. Bouwman, K. Kjaer, and H. Möhwald. 1995b. Influence of ether linkages on the structure of double-chain phospholipid monolayers. *Chem. Phys. Lipids.* 76:145–157.

Brezesinski, G., M. Thoma, B. Struth, and H. Möhwald. 1995c. Structural changes of monolayers at the air/water interface contacted with alkanes. *J. Phys. Chem.* In press.

Dietrich, A., G. Brezesinski, H. Möhwald, B. Dobner, and P. Nuhn. 1994. Domain shapes and monolayer structures of triple-chain phospholipids on water. *Il Nuovo Cimento.* 16:1537–1544.

Dietrich, A., H. Möhwald, W. Rettig, and G. Brezesinski. 1991. Polymorphism of a triple-chain lecithin in two- and three-dimensional systems. *Langmuir.* 7:539–546.

Jacquemain, D., S. Grayer-Wolf, F. Leveiller, M. Deutsch, K. Kjaer, J. Als-Nielsen, M. Lahav, and L. Leiserowitz. 1992. Zweidimensionale Kristallographie an amphiphilen Molekülen an der Luft-Wasser-Grenzfläche. *Angew. Chemie.* 104:134–158.

Jaques, J., A. Collet, and S. H. Wilen. 1981. Enantiomers, Racemates and Resolutions. Wiley, New York.

Kaganer, V. M., and E. B. Loginov. 1995. Symmetry and phase transitions in Langmuir monolayers: The Landau theory. *Phys. Rev. E* 51: 2237–2249.

Kaganer, V. M., M. A. Osipov, and I. R. Peterson. 1993. A molecular model for tilting phase transitions between condensed phases of Langmuir monolayers. *J. Chem. Phys.* 98:3512–3527.

Kaganer, V. M., I. R. Peterson, R. M. Kenn, M. C. Shih, M. Durbin, and P. Dutta. 1995. Tilted phases of fatty acid monolayers. *J. Chem. Phys.* 102:9412–9422.

Kenn, R. M., C. Böhm, A. M. Bibo, I. R. Peterson, H. Möhwald, J. Als-Nielsen, and K. Kjaer. 1991. Mesophases and crystalline phases in fatty acid monolayers. *J. Phys. Chem.* 95:2092–2097

Kjaer, K. 1994. Some simple ideas on x-ray reflection and grazing-incidence diffraction from thin surfactant films. *Phys. B.* 198:100–109.

Kjaer, K., J. Majewski, H. Schulte-Schrepping, and J. Weigelt. 1992. Commissioning and first results from the liquid surface diffractometer BW1 in HASYLAB. *HASYLAB Annu. Rep.* 589–590.

Lewis, R. N. A. H., R. N. McElhaney, P. E. Harper, D. C. Turner, and S. M. Gruner. 1994. Studies of the thermotropic phase behavior of phosphatidylcholines containing 2-alkyl substituted fatty acyl chains: a new class of phosphatidylcholines forming inverted nonlamellar phases. *Biophys. J* 66:1088–1103.

Moy, V. T., D. J. Keller, and H. M. McConnell. 1988. Molecular order in finite two-dimensional crystals of lipid at the air-water interface. *J. Phys. Chem.* 92:5233–5238.

Nassoy, P., M. Goldmann, O. Bouloussa, and F. Rondelez. 1995. Spontaneous chiral segregation in bidimensional films. *Phys. Rev. Lett.* 75: 457–460.

Nuhn, P., G. Brezesinski, B. Dobner, G. Förster, M. Gutheil, and H.-J. Dörfler. 1986. Synthesis, calorimetry, and x-ray diffraction of lecithins containing branched fatty acid chains. *Chem. Phys. Lipids.* 39:221–236.

Paltauf, F., and A. Hermetter. 1994. Strategies for the synthesis of glycerophospholipids. *Prog. Lipid Res.* 33:239–328.

Parazak, D. P., J. Y.-J. Uang, B Turner, and K. J. Stine. 1994. Fluorescence microscopy study of chiral discrimination in Langmuir monolayers of *N*-acyl valine and alanine amphiphiles. *Langmuir.* 10:3787.

Rattay, B., G. Brezesinski, B. Dobner, G. Förster, and P. Nuhn. 1995. Influence of α-branched fatty acid chains on the thermotropic behaviour of racemic 1-*O*-hexadecyl-2-acyl-glycero-3-phosphocholines. *Chem. Phys. Lipids.* 75:81–91.

Scalas, E., G. Brezesinski, H. Möhwald, V. M. Kaganer, W. G. Bouwman, and K. Kjaer. 1995. Chirality effects on 2D phase transitions. *Thin Solid Films.* In press.

Stine, K. J., J. Y.-J. Uang, and S. D. Dingman. 1993. Comparison of enantiomeric and racemic monolayers of *N*-stearoylserine methyl ester by fluorescence microscopy. *Langmuir.* 9:2112–2118.

Struth, B., E. Scalas, G. Brezesinski, H. Möhwald, F. Bringezu, W. G. Bouwman, and K. Kjaer. 1994. Influence of a hydrophilic spacer on the structure of a phospholipid monolayer. *Il Nuovo Cimento.* 16: 1545–1550.

Viswanathan, R., J. A. Zasadzinski, and D. K. Schwartz. 1994. Spontaneous chiral symmetry breaking by achiral molecules in a Langmuir-Blodgett film. *Nature (Lond.).* 368:440–443.

Weiss, R. M., and H. M. McConnell. 1984. Two-dimensional chiral crystals of phospholipid. *Nature (Lond.).* 310:47–49.