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The amphiphilic drug flufenamic acid can induce a hexagonal phase in DMPC: a solid state ^{31}P- and ^{19}F-NMR study

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Received 16th May 2000, Accepted 24th July 2000

First published as an Advance Article on the web 23rd August 2000

Established solid state ^{31}P-NMR and novel ^{19}F-NMR experiments are used in a complementary approach to describe the behaviour of a fluorinated drug, flufenamic acid (FFA), in phospholipid model membranes. The non-steroidal anti-inflammatory agent FFA was dissolved at 5% (w/w) in dimyristoylphosphatidylcholine (DMPC), and the system was investigated at low hydration (3 H_2O per lipid) where morphological transitions of the lipid are strongly affected by additives. It is demonstrated that FFA induces a fluid H_{II} phase in DMPC at ambient temperatures, *i.e.* much below its regular chain-melting transition which occurs around 50 °C at low hydration. The guest molecules are preferentially accommodated in the hexagonal phase of the lipid, which coexists with the usual crystalline state of pure DMPC. The peculiar transition sequence $L_C \rightarrow H_{II} \rightarrow L_\alpha$ with increasing temperature is explained by a re-distribution of FFA in the lipid matrix and a concomitant phase separation under conditions of limiting hydration. Small-angle X-ray diffraction and freeze–fracture electron microscopy are used to confirm the existence of the hexagonal and bilayer phases, and to determine their respective dimensions. When the drug FFA dissolves in the bilayer, its structural effect on the surrounding lipid molecules may be related to its pharmacological activity in membranes. For example, FFA is known to modulate ion channel function, and it has been suggested that it inhibits phospholipase activity by accelerating the transbilayer flip-flop of lipids.

Introduction

Flufenamic acid (FFA) belongs to the class of non-steroidal anti-inflammatory drugs, which are clinically used against a wide range of acute and chronic disorders. The molecular basis for its therapeutic action is generally believed to be its ability to inhibit cyclooxygenase activity and thereby block the production of prostaglandins.[1] An unrelated, additional mode of anti-inflammatory action of FFA has been recently attributed to its inhibition of Ca^{2+} influx into activated neutrophils,[2,3] and to the corresponding arrest of Ca^{2+}-dependent lymphocyte proliferation.[4] In this scenario it appears that ion channels are the target of the drug. Indeed, FFA is known to inhibit various non-selective cation channels,[5,6] chloride channels,[7] an organic anion transporter[8] and it binds to the erythrocyte anion exchanger band-3.[9]

Flufenamic acid can be accommodated in both polar and apolar environments, depending on the protonation state of its carboxylic acid group which has a pK_a around 4–5. It has been suggested that the presence of FFA exerts a significant influence on the physical state of the lipid bilayer and thereby modulates the function of membrane-associated proteins, for example of phospholipase A_2.[10] For example, it was demonstrated that lipid-soluble compounds like FFA inhibit phospholipase *in vitro* by accelerating the transbilayer flip-flop of lipids and lysolipids.[11] When the compounds were tested whether they could induce inverted lipid phases in phosphatidylcholine, no such intermediates were detected under ambient conditions. Here, we demonstrate under more rigorous conditions of low hydration[12] that FFA is indeed able to induce an abundant hexagonal phase in DMPC. Solid-state NMR, small-angle X-ray diffraction (SAXS) and electron microscopy (EM) are used as complementary methods to examine the lipid morphology.[13,14]

Besides relying on established ^{31}P-NMR methods to determine the phase state of a phospholipid dispersion,[15–17] it is also useful to observe a guest compound directly by NMR.[18–21] Flufenamic acid, like many other pharmacologically active drugs and anaesthetics, contains fluorine which is a highly sensitive NMR probe.[22,23] We have recently analyzed the ^{19}F-NMR signals of FFA to assess its mobility and alignment in DMPC model membranes, by measuring the homonuclear dipolar coupling within the CF_3-group.[24] In that study, which was meant to illustrate the use of uniaxially oriented NMR samples for determining the orientation-dependence of the ^{19}F dipolar coupling, the first signs of a non-lamellar lipid phase were noted:[25] At full hydration FFA resided in lamellar bilayers as expected, but at low water content it revealed a different morphology which was tentatively assigned to be hexagonal. Here, we investigate the FFA–DMPC mixture at low hydration in more detail in order to verify the induction of an inverted H_{II} phase at ambient temperature. No such tendency has been observed previously in pure phosphatidylcholines by X-ray diffraction,[26,27] FTIR-

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 DOI: 10.1039/b003902k

ATR spectroscopy,[28] electron microscopy,[29] differential scanning calorimetry[30] or by solid-state NMR.[31] The disturbing effect of the pharmacologically active drug observed here thus supports the notion that it may indeed facilitate rapid transbilayer flip-flop in the membrane,[11] as it had been postulated earlier to explain the inhibition of phospholipase A_2.[11] It remains to be shown whether any of the other membrane proteins which respond to FFA could also be mediated in a similar manner *via* non-specific binding of the drug to the lipid matrix.

Materials and methods

Samples were prepared by co-dissolving DMPC (Avanti Polar Lipids, Alabaster, USA) and flufenamic acid (Sigma-Aldrich) at 5% (w/w) in $CHCl_3$. After evaporation and overnight vacuum, the material was hydrated with a well-defined amount of doubly distilled water using a Hamilton syringe. Assuming that one molecule of H_2O had remained bound to each lipid, an accuracy of $n_W \pm 0.5$ H_2O (mol/mol) could be achieved, where n_W denotes the number of H_2O per DMPC molecule. Oriented samples for ^{19}F-NMR were prepared on glass plates as previously described.[24,25] The reproducibility of the NMR spectra was checked by incubating several freshly hydrated samples above their chain melting transition ($T_m \approx$ 50 °C) and subsequently equilibrating them again at the desired measuring temperature below T_m.[29] It was thereby confirmed that any morphological transitions (especially $L_\alpha \rightarrow L_C$) had reached their equilibrium state after a sufficiently long period of time (several minutes up to many hours), and that the observed structures were not trapped in any metastable gel phase.

Solid-state NMR measurements were performed on a Unity Inova 500 MHz Varian spectrometer, at a resonance frequency of 202.4 MHz for ^{31}P and 470.4 MHz for ^{19}F. Hahn-echo ^{31}P-NMR spectra were acquired with ^{1}H-decoupling and 4 μs 90° pulse lengths and 30 μs delays. Single-pulse ^{19}F-NMR spectra were acquired with ^{1}H-decoupling and pulse lengths of 2.5 μs, while the Carr–Purcell–Meiboom–Gill (CPMG) sequence employed 20 μs windows and composite 90°–180°–90° pulses without ^{1}H-decoupling.[24,25] Temperature-dependent experiments were carried out in the range between 15 and 55 °C, controlled to within an accuracy of about 2 °C. Lineshape simulations were carried out as described earlier.[25,32,33]

X-Ray diffraction studies were performed at the EMBL outstation at DESY in Hamburg, using synchrotron radiation from the storage ring DORIS III in combination with the X33 double-focussing monochromator-mirror camera.[34] Samples prepared the same way as for NMR were placed in standard liquid cells supplied by EMBL. About 100 μl of the aqueous lipid dispersion were squeezed between the mica plates of these cells having a pathlength of *ca.* 1 mm. Significant deviations from the pre-adjusted water content ($n_W = 3$) are highly improbable when taking into account that the material was rapidly transferred at room temperature under ambient relative humidity (RH). Adsorption isotherms of water on DPPC[35] and DMPC (D. R. Gauger and W. Pohle, unpublished data) determined previously by IR spectroscopy had demonstrated that the hydration level at ambient RH closely corresponds to $n_W = 3$–4. The X-ray measurements were carried out over a range of temperatures between 5 and 85 °C. Data were processed using the OTOKO program.

Freeze–fracture electron microscopy replicas were obtained from the same FFA–DMPC dispersion as above, which was fast-frozen from 35 °C, as described previously.[29] Fracturing and replication were carried out with a BAF 400 T freeze–fracture device (Balzers, Liechtenstein), and the replicas were examined in an EM 900 electron microscope (Zeiss, Germany).

Results and discussion

Flufenamic acid (FFA, see inset of Fig. 1) readily partitions into cellular membranes, even when added in its deprotonated state at micromolar concentrations from aqueous buffer at physiological pH (J. Salgado and A. S. Ulrich, unpublished results). We showed in a previous NMR study that multilamellar liposomes containing 5% (w/w) FFA retain their bilayer structure, provided that the lipid is fully hydrated.[25] However, we noted that FFA affects the lipid morphology at low hydration, which is examined more closely in Fig. 1. The left column shows ^{31}P-NMR spectra of DMPC containing FFA, hydrated with $n_W = 2$, 3, 4 and 5 H_2O molecules per lipid (Fig. 1a–d). At 35 °C these successive hydration levels are expected to induce a transition from a crystalline L_C state to the fluid L_α phase in DMPC.[27,28] This transition is confirmed in the pure DMPC controls on the right (Fig. 1e–h). The static ^{31}PCSA lineshape which dominates at low hydration is assigned to a crystalline L_C phase, because the three principal tensor values are clearly resolved. With increasing hydration, the L_C phase of pure DMPC is gradually replaced by an axially symmetric lineshape, being characteristic of a fluid L_α phase (Fig. 1h).

In the presence of FFA, on the other hand, the sample with $n_W = 2$ shows an isotropic peak at 0 ppm in coexistence with the L_C signal (Fig. 1a). At $n_W = 3$ a reversed axially symmetric lineshape is observed (Fig. 1b) which contributes about 20% to the total intensity. Only at higher hydration levels the usual L_α phase emerges in the FFA-containing samples (Fig. 1c,d). The isotropic ^{31}PNMR signal (Fig. 1a) cannot be interpreted unambiguously, but it is compatible with a cubic or inverted micellar phase of DMPC. The reversed axially symmetric signal at $n_W = 3$ (Fig. 1b), however, is characteristic of a hexagonal phase.[14–17] In view of the reduced hydration shell around the lipid headgroups, we attribute this phase to an inverted H_{II} type. Although inverted lipid phases are not at all typical for phosphatidylcholines, they have been observed at temperatures above 100 °C in pure DMPC and DPPC under

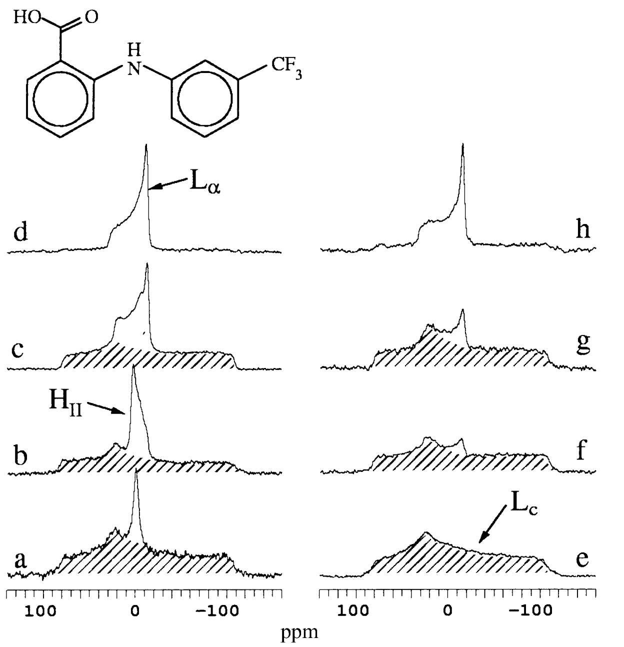

Fig. 1 ^{31}P-NMR spectra of DMPC containing 5% FFA (top inset) (a–d), and of pure DMPC (e–h). The hydration level is varied from $n_W = 2$ (a,e), 3 (b,f), 4 (c,g) to 5 (d,h) water molecules per lipid, at 35 °C. Both samples undergo a lyotropic phase transition from a crystalline L_C (broad, hatched component) to a fluid L_α state (axially symmetric averaged ^{31}P-NMR lineshape with peak to the right of 0 ppm). Additionally, a hexagonal phase is formed in the FFA–DMPC mixture at a hydration level of around $n_W = 3$ (narrow lineshape with peak to the left of 0 ppm).

conditions of low hydration.[26–28] H_{II} phases have even been reported at ambient temperature for mixtures of DMPC with fatty acids[36] or with gramicidin A[37] at high solute concentrations. Likewise, the current study shows that 5% of FFA is sufficient to induce a considerable negative curvature in the DMPC matrix when aided by the low hydration level of the lipid headgroups.[12]

The effect of raising the temperature of the FFA–DMPC mixture containing the H_{II} phase (Fig. 1b, with $n_W = 3$, at 35 °C) is illustrated in Fig. 2. Up to 45 °C (Fig. 2a,b) the H_{II} signal increases linearly to about 33% of the total intensity. At 50 °C (Fig. 2c) a new fraction of an L_α phase emerges additionally, and at 55 °C (Fig. 2d) the whole sample has been converted into fluid bilayers. This sequence is fully reversible back to 35 °C and requires only a few minutes to regain its equilibrium state, which we noted to be much faster than the corresponding $L_\alpha \rightarrow L_\beta \rightarrow L_C$ transition in pure DMPC which takes several hours. Compared to the main phase transition temperature T_m of fully hydrated DMPC at 23 °C, the substantial dehydration-induced shift by about 30 °C is plausible in view of an empirical relationship previously reported for DMPC.[38] It has thus been demonstrated that *below* the regular phase transition of DMPC (which occurs at around 50 °C at low hydration), FFA is able to induce an H_{II} phase with fluid lipid chains, in coexistence with the usual crystalline L_C phase. Such coexistence has so far only been described for diacyl- and dialkyl-phosphatidylethanolamines.[39]

The question arises next, whether all the FFA guest molecules are accumulated in the H_{II} phase or whether they are distributed throughout the lipid matrix. This point is addressed by ^{19}F-NMR measurements on FFA, providing a complementary view on the mixed FFA–DMPC system.[22,25] Fig. 3 shows ^{19}F-NMR spectra of 5% FFA in DMPC (n_W = 3), below (Fig. 3a,c) and above (Fig. 3b,d) the phase transition temperature T_m of around 50 °C. The standard ^{1}H-decoupled ^{19}F-NMR spectra of the lipid dispersion (Fig. 3a,b) are dominated by both the chemical shift and the homonuclear dipolar interactions of the CF_3-group. The lineshape below T_m (Fig. 3a) is significantly narrower than above (Fig. 3b). This qualitative difference is consistent with the expected additional motional narrowing in the putative H_{II} phase due to molecular diffusion around the cylinder axes. The splitting in the powder spectrum above T_m (Fig. 3b) reflects the homonuclear dipolar coupling within the CF_3-group, but the contributing lines are not well resolved.

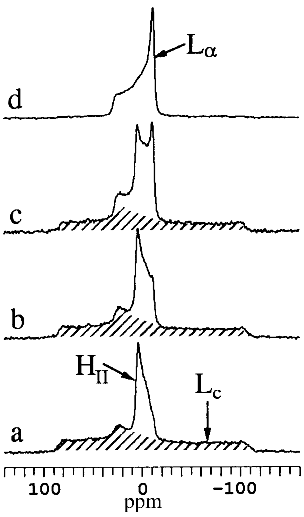

Fig. 2 ^{31}P-NMR spectra of DMPC containing 5% FFA, hydrated with $n_W = 3$ and acquired at temperatures of 40, 45, 50 and 55 °C (a–d). The lipid reaches its main phase transition at around T_m = 50 °C, where the crystalline L_C phase (broad, hatched component) changes into fluid L_α bilayers. Below T_m, an additional hexagonal H_{II} phase coexists with L_C.

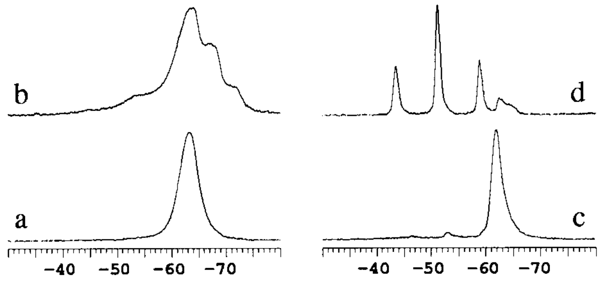

Fig. 3 ^{19}F-NMR single-pulse spectra of 5% FFA in DMPC, prepared as non-oriented (a,b) and oriented (c,d) samples at low hydration (n_W = 3). Below the transition temperature T_m of around 50 °C, the narrow featureless spectra (a,c) are consistent with a high degree of motional averaging in a hexagonal phase. Above T_m, on the other hand, the chemical shift and dipolar coupling anisotropies dominate the spectral lineshapes (b,d). When a macroscopically oriented sample is prepared and aligned in the magnetic field, the spread of anisotropies is removed and the lines are better resolved (d).

Better resolution in the ^{19}F-NMR spectra is available from uniaxially oriented membranes on glass plates, since a unique value is selected from the range of the chemical shift anisotrophy and of the dipolar coupling are reduced to a unique value when the sample normal is aligned parallel ($\alpha = 0°$) to the static magnetic field direction. The spectrum below T_m (Fig. 3c) is much narrower than the triplet at high temperature (Fig. 3d), which is again consistent with additional motional averaging of FFA in an H_{II} phase. However, it appears that the local tumbling of FFA, as defined by its residual alignment with respect to the neighbouring lipids, occurs around a different molecular axis (or to a different extent) in the two phases. If the molecules in the H_{II} phase were only being averaged by an additional rotation at right angle around the cylinder axes[17] (besides their long-axial rotation), the dipolar splittings of the two phases would differ exactly by a factor of $[\cos^2(90°) - 1]/2 = -1/2$. Instead, the dipolar splitting in the L_α phase is more than twice as large as the unresolved splitting of the putative H_{II} phase.

The ^{19}F-NMR data alone have not yet provided direct evidence for a hexagonal or lamellar environment of FFA. Nevertheless, they have proven that all FFA molecules experience the same lipid environment at temperatures approaching T_m, since the ^{19}F-NMR lineshape reflects a single population. The transition temperature observed by ^{19}F-NMR also coincides with the $L_C + H_{II} \rightarrow L_\alpha$ transition described for the phospholipid matrix by ^{31}P-NMR (Fig. 2). Since the entire ^{19}F signal intensity is reshaped by the transition at T_m, we conclude that the guest molecules are preferentially accommodated in the H_{II} phase rather than in the L_C phase. Given that 5% (w/w) of FFA correspond to a molar lipid : solute ratio of about 8 : 1, and having shown that up to 33% of the lipid molecules are converted to the H_{II} phase, this means that the local lipid:solute ratio in the hexagonal cylinders is about 3 : 1. This high local concentration of FFA makes the formation of an inverted phase plausible, and the low water content is furthermore expected to favour phase separation.[40]

Further resolution enhancement in static ^{19}F-NMR spectra of coupled spins can be achieved by combining the use of oriented samples with a modified CPMG experiment.[19,24] CPMG-type spectra of the same oriented sample as above (5% FFA in DMPC, n_W = 3) are shown at temperatures below (Fig. 4a) and above T_m (Fig. 4b). Since the multipulse sequence removes any contributions from the chemical shift and selects only the homonuclear dipolar couplings, even the small dipolar splitting of 320 Hz in the putative H_{II} phase is visible (Fig. 4a). Above T_m (Fig. 4b) the large splitting of 3600 Hz is the same as in the single-pulse experiment (Fig. 3d), but now the lines are much sharper. Based on these well resolved

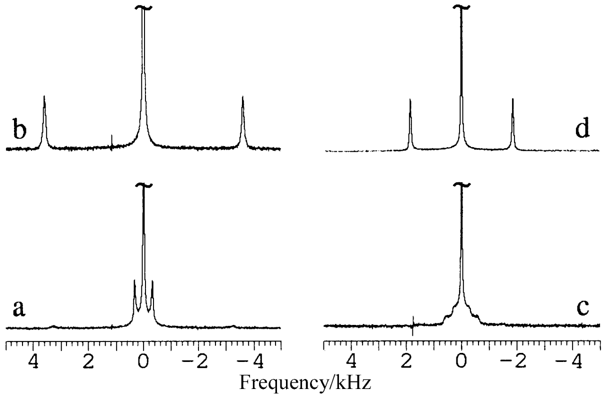

Fig. 4 ^{19}F-NMR spectra of 5% FFA in DMPC prepared as an oriented sample at low hydration ($n_W = 3$), acquired with a modified CPMG experiment. With the aid of multipulse narrowing the dipolar couplings are much better resolved (a,c) than in the conventional spectra (*cf.* Fig. 3). The dipolar coupling in the hexagonal phase below T_m (a, 320 Hz) is smaller than in the lamellar phase above T_m (b, 3600 Hz). By changing the sample orientation (α = angle of the sample normal with respect to the magnetic field) from $\alpha = 0°$ (a,b) to $\alpha = 90°$ (c,d), it is possible to determine the axis around which FFA is being motionally averaged. We find that below the T_m this axis is aligned in the plane of the sample (a,c), and above T_m it is oriented perpendicular to the macroscopic support (b,d).

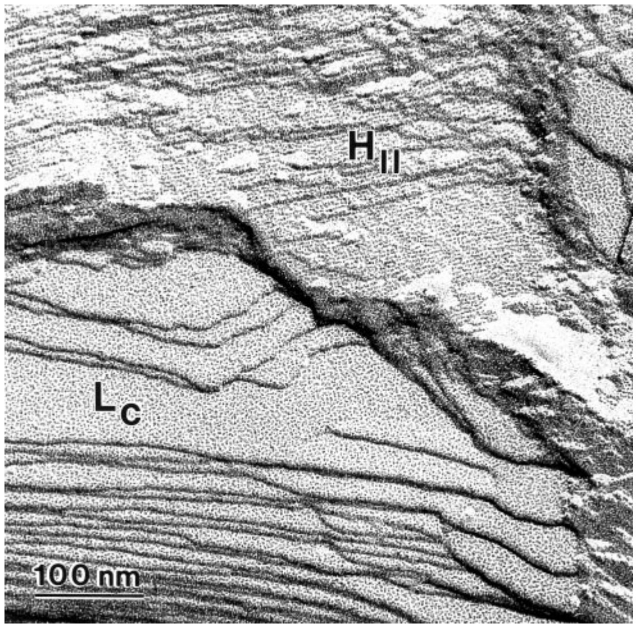

Fig. 6 Freeze–fracture EM of DMPC containing 5% FFA, hydrated with $n_W = 3$ and quenched from 35 °C. It shows lamellar fracture faces (L_C) in coexistence with bundles of hexagonally packed cylinders (H_{II}).

spectra, it is now possible to carry out an orientation-dependent lineshape analysis of the macroscopically aligned sample, as illustrated in Fig. 4c and d. By tilting the sample in the magnetic field, the resulting changes in the lineshape can be used to determine the angle between the molecular axis of averaging and the sample normal, as described previously.[32,33] The oriented ^{19}F-NMR spectra of FFA in DMPC are shown in Fig. 4a and b where the sample normal is aligned parallel ($\alpha = 0°$) to the magnetic field direction. Comparison with the perpendicular sample alignment at $\alpha = 90°$ (Fig. 4c and d) demonstrates that the orientation of the molecular axis differs for the two different temperatures. Lineshape analysis shows that, below the phase transition, the FFA molecules are motionally averaged around cylindrical axes which are aligned *in-plane* of the sample.[25] This geometrical interpretation is fully consistent with the hexagonal

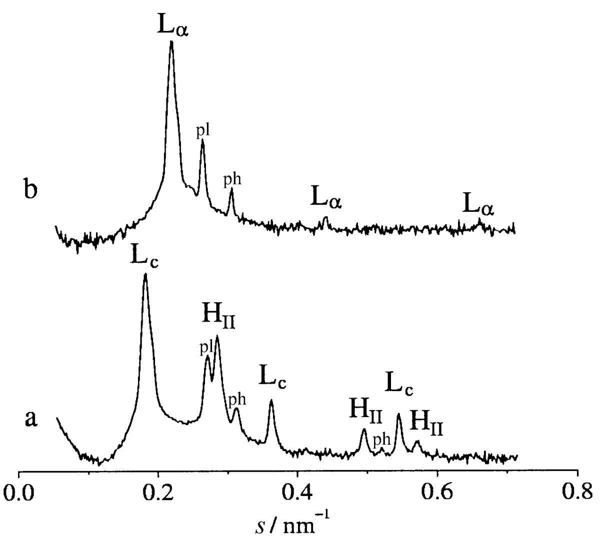

Fig. 5 Logarithmic plot of SAXS amplitudes of DMPC containing 5% FFA, hydrated with $n_W = 3$, recorded below (a) and above (b) the phase transition temperature. Below T_m, a lamellar L_C phase coexists with a hexagonal H_{II} phase. Above T_m, both phases are converted into a fluid L_α phase. Small amounts of further putative hexagonal (ph) and putative lamellar (pl) phases are noted.

lipid phase suggested above by ^{31}P-NMR (Fig. 1 and 2). Above T_m on the other hand, the FFA molecules are averaged around the macroscopic sample normal, as expected for the liquid-crystalline environment of the lamellar L_α phase.

In parallel to the NMR experiments, SAXS and freeze–fracture EM were used to confirm the occurrence of a hexagonal phase under appropriate conditions. A temperature-dependent SAXS scan between 5 and 85 °C of an FFA–DMPC dispersion with $n_W = 3$ H_2O revealed similar structural changes as observed by NMR. At temperatures well below the overall chain-melting transition (*e.g.* at 15 °C, data not shown), most of the sample exists in a lamellar phase with a repeat distance of about 55 ± 0.3 Å (Fig. 5a), which is identical to that of crystalline DMPC dihydrate.[26] The corresponding wide-angle diffraction data reveal three sharp reflexes due to the acyl chain packing, hence we identify this phase as L_C, in agreement with the ^{31}P-NMR data (Fig. 1 and 2). Approaching the main phase transition (which is observed here at $T_m = 55$ °C), an additional hexagonal phase emerges, which is clearly specified by the 2nd and 3rd order reflexes at 0.494 and 0.543 nm^{-1} (Fig. 5a). Its lattice constant of 40.6 Å is in reasonable accord with literature data on related lipids.[39] In the SAXS pattern obtained above T_m (Fig. 5b), the hexagonal pattern has disappeared and the repeat distance of the lamellar phase is decreased to about 45 Å. The intensity of the higher-order reflexes of the lamellar phase is significantly reduced, thus confirming the formation of a fluid L_α phase. Its repeat spacing agrees essentially with the diffraction data reported on DMPC in excess water.[41] The two weak reflexes at 0.31 and 0.27 nm^{-1} could not be unambiguously assigned, and their coexistence with the other phases may reflect a certain degree of sample inhomogeneity. Their signatures are consistent with a second putative hexagonal and another putative lamella phase, which we termed ph and pl, respectively, but any such interpretation has to be treated with great caution. (The 0.31 nm^{-1} peak could be correlated with a very weak 2nd order reflex near 0.53, *i.e.* in a $\sqrt{3}$ distance; the 0.27 nm^{-1} peak on the other hand has no accompanying $\sqrt{3}$ reflex, but it could have a lamellar 2nd order reflex at 0.54 nm^{-1} superimposed by the 3rd order peak of the L_C phase.) In summary, the X-ray data have confirmed the coexistence of the lamellar and hexagonal lipid phases suggested by ^{31}P- and

^{19}F-NMR, and their respective repeat distances are fully consistent with the expected dimensions.

Freeze–fracture electron micrographs were obtained from the original NMR samples, by rapidly quenching from 35 °C. The images in Fig. 6 reveal typical hexagonal structures in coexistence with flat lamellar fracture faces, thus confirming the solid-state NMR and X-ray data. We estimated the repeat distance of the hexagonal patterns from the EM images to be approximately 4 nm, which is in good agreement with the X-ray results.

Conclusion

It has been demonstrated that the anti-inflammatory drug flufenamic acid is able to induce a hexagonal lipid phase in DMPC at ambient temperatures under conditions of low hydration ($n_W = 3$ H_2O). Solid-state ^{31}P-NMR was used to describe the different phases of the lipid matrix, ^{19}F-NMR revealed the behaviour of the guest compound, and the sample morphology was examined by X-ray diffraction and EM. These complementary methods yielded a consistent picture, summarized in Fig. 7, where two coexisting $L_C + H_{II}$ lipid phases are converted into an L_α phase at around 50 °C. At ambient temperatures all FFA guest molecules are accommodated within the H_{II} cylinders of DMPC, although at very low temperatures they, too, seem to be adapted to an immobile L_C state. Several aspects of these observations are noteworthy: (A) Hexagonal phases do not usually occur in pure phosphatidylcholines below 100 °C,[26,28] and they are only rarely observed in mixtures of DMPC with other components.[36,37] (B) A coexistence of fluid and crystalline domains has so far only been reported for ethanolamines.[39] (C) The unusual phase transition sequence $L_C \rightarrow H_{II} \rightarrow L_\alpha$ can be explained by a local phase separation into FFA-rich and FFA-depleted domains.[40] Taken together, it appears that the resulting high local concentrations of FFA strongly affect the lipid–lipid interactions of DMPC, inducing a negative curvature in the lipid matrix and leading to chain melting. FFA must therefore be accommodated in the hydrophobic region between the acyl chains, which is consistent with the protonated state of this organic acid.

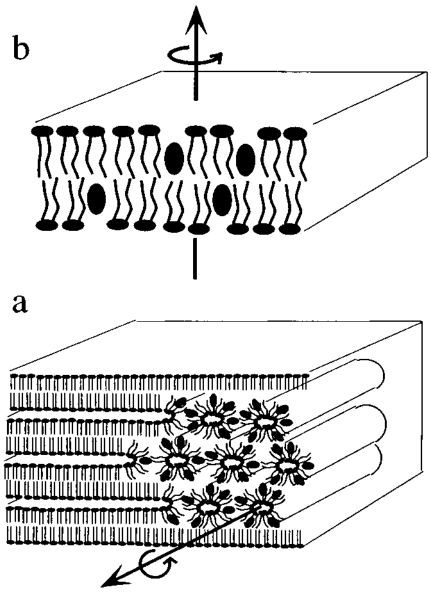

Fig. 7 Model of the behaviour of FFA in DMPC at low hydration. At low temperature (a), 5% FFA are sufficient to induce the formation of a fluid H_{II} phase in about 1/3 of the lipid matrix. FFA is preferentially incorporated into the H_{II} cylinders, while the remaining lipid persists in the crystalline L_C bilayers. At high temperature (b), all lipid chains are melted and the sample assumes a uniform L_α phase.

In addition to its generally accepted target cyclooxygenase,[1] FFA has been previously shown to modulate the function of various membrane-associated proteins, such as ion channels[2–7] and transporters.[8,9] Besides binding to specific sites on membrane proteins, the high lipid solubility of FFA raises the question whether it might exert further indirect effects on these proteins *via* the bilayer. For example, the inhibition of phospholipase A_2 by FFA[10] has been attributed to a solute-induced accelerated transbilayer flip-flop of the lipids.[11] Indeed, our observation of an FFA-induced H_{II} phase in DMPC lends support to this idea,[42] since the transmembrane exchange process is likely to involve inverted lipid phases as intermediates.[14,16,43,44] FFA is obviously not the only small solute that can accelerate such exchange, but besides its intrinsic pharmaceutical activity the drug benefits from its easy access to targets that are located both in the cell membrane and in the cytosol. When the amphiphilic FFA molecules are administered orally or topically in aqueous solution, they appear to be able to diffuse across and accumulate in cellular membranes, and to traverse the stratum corneum or potentially even the blood-brain barrier.

Acknowledgements

We thank Dr. M. Koch (EMBL outstation at DESY in Hamburg) for guiding the SAXS measurements and HASYLAB for financial support. The DFG is gratefully acknowledged for promoting this cooperation between projects TP A1, B10 and B13 within the SFB 197.

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