Binding of Nonsteroidal Anti-inflammatory Drugs to DPPC: Structure and Thermodynamic Aspects

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The effect of nonsteroidal anti-inflammatory drugs (NSAIDs) on the phase transition and phase properties of 1,2-dipalmitoylphosphatidylcholine (DPPC) has been investigated in both 2D (monolayers at the air/water interface) and 3D (multilayers in lipid/water dispersions) model systems. The 2D membrane models have been characterized by means of pressure—area isotherms and grazing incidence X-ray diffraction (GIXD) measurements. Differential scanning calorimetry (DSC) and simultaneous small- and wide-angle X-ray diffraction have been applied to lipid aqueous dispersions. All NSAIDs studied altered the main transition temperature of the gel to liquid-crystalline phase transition, with the arylacetic acid derivatives (acemetacin and indomethacin) showing the largest effects. A comparison of the results reveals distinct structural features of the membrane models after interaction with the NSAID. All drugs induced perturbations in the lipid liquid-crystalline phase, suggesting a major change in the hydration behavior of DPPC. Again, the largest effects on the structural parameters are found for the arylacetic acid derivatives. The results obtained in the different model systems give indications of the role of the membrane/NSAID interactions that might also be important for NSAID gastric injury.

Introduction

Cyclooxygenase (COX), also known as prostaglandin synthase (PGH), is a potent mediator of inflammation.1 Nonsteroidal anti-inflammatory drugs (NSAIDs) bind to cyclooxygenase, thereby inhibiting the production of prostaglandins. COX-1 and COX-2 are two isoforms of cyclooxygenase.2 COX-1 is a constitutive enzyme that produces gastroprotective prostaglandins,3 and COX-2 is induced by cytokines, mitogens, and endotoxins in inflammatory cells, which are responsible for the production of inflammatory prostaglandins.4 Most of the NSAIDs bind at the active sites of both COX-1 and COX-2 with little specificity and lead to side effects such as gastric lesions and renal toxicity.

The discovery by Vane and colleagues provided new insight into both the mechanisms of NSAID therapeutic activities and side effects1,2,5–7 such as the development of inhibitors that selectively bind to COX-2, which would be expected to show anti-inflammatory action in vivo with minimal gastric side effects. However, the sequence of events resulting from cyclooxygenase inhibition does not completely explain the overall gastric toxicity of NSAIDs. The development of novel NSAIDs showing less serious side effects during medical applications will also depend on the understanding of the processes initiating and promoting gastric injury. Such mechanisms are complex, and the cascade of events leading to mucosal damage must therefore be characterized and can also be related to the topical irritancy of NSAIDs. Davenport suggested that the normal resistance of the gastric mucosa to back diffusion of luminal acid can be disrupted by the topical administration of lipid-soluble damaging agents such as acetylsalicylic acid or aspirin.8 Evidence of the direct superficial damaging effects of other drugs that are members of the NSAID family was subsequently provided by many investigators who showed histological, biochemical, and permeability changes in the gastric mucosa.9–11 However, the “barrier-breaking” activity of the drugs has not been established on a molecular basis.

Despite the complexity of the acid resistance properties of the gastric inner surface, the extracellular lining of surfactant-like phospholipids on the surface within the mucus gel layer represents an initial line of defense of the stomach and confers hydrophobic, non-wettable, acid-resistant properties to the mucosa.12 NSAIDs appear to decrease mucosal hydrophobicity because of their ability to suppress prostaglandin synthesis. In addition, they may chemically associate with phospholipids and destabilize them from the mucus gel layer.13 Such a transition would increase the wettability of the stomach and result in an increase in the back-diffusion of luminal acid into the mucosa; consequently, the development of erosions must be expected. The aim of the

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present study was therefore to investigate the ability of NSAIDs
to bind to or to penetrate into layers of surface-active phos-
pholipids. The results may elucidate the effects of these drugs
compromising the integrity of the gastric mucosal barrier. For
this purpose, three different NSAIDs (indomethacin, acemetacin,
and nimesulide; Figure 1) were selected, and their influence on
the biophysical properties of 1,2-dipalmitoylphosphatidylcholine
(DPPC) was studied by different complementary experimental
techniques in 2D and 3D model systems.

DPPC is a typical phospholipid with regard to its role in
determining the physical—chemical and biological properties of
cellular membranes.14 By dispersing this lipid in water, multi-
lamellar vesicles are spontaneously formed that exhibit many
features of the cellular membranes (e.g., the barrier and transport
function or permeability by passive diffusion through the bilayer).
In aqueous dispersions, DPPC exhibits a rich polymorphism that
encompasses crystalline, gel, and liquid-crystalline phases. The
formation of these structures depends on the environmental
conditions (e.g., temperature, pressure, salt conditions, vesicle
preparation, and hydration).15–16 According to this, in the current
work differential scanning calorimetry measurements (DSC)
were applied for the thermoanalysis of the phase behavior of the lipid
aqueous dispersions and the effect of the NSAID on the phase-
transition parameters. The results are compared with structural
information from small- and wide-angle X-ray scattering (SAXS/
WAXS)17 to learn about the influence of the NSAID on the
structures of the pure DPPC dispersion.

The interaction of the NSAID with lipid bilayers must be
considered to be a complex event of adsorption, penetration,
and translocation that involves a change in the lipid hydration
and molecular orientation of both the lipid head groups and the chains
forming the hydrophobic membrane core. Using Langmuir
monolayers at the air/liquid interface allows one to separate the
adsorption and penetration of the NSAID toward the membrane
surface from the trans-bilayer events.18 Special interest in using
phospholipid monolayers for membrane studies arises from the
possibility of varying the physical—chemical parameters at the
interface, such as molecular density, lateral pressure, surface
potential, and ionic conditions. In particular, in the study described
here, the option to manipulate the subphase was used to allow
drug adsorption and penetration into the lipid film present at the
interface. Structural changes within the DPPC monolayer upon
adsorption of indomethacin were investigated by synchrotron
X-ray experiments at grazing incidence (GIXD). These experi-
ments were carried out to obtain information on the structure of
the DPPC monolayer interacting with indomethacin. The effect
of NSAIDs on the lipid organization in 2D monolayers and 3D
bilayers are investigated and compared.

**Experimental Methods**

**Materials.** DPPC, indomethacin, acemetacin, and nimesulide were
obtained from Sigma Chemical Co. (St. Louis, MO), and used without
further purification. All other chemicals were purchased from Merck
(Darmstadt, Germany). Solutions were prepared with HEPES buffer
(10 mM, 0.1 M NaCl, pH 7.4). Water was purified with a Millipore
desktop system, leading to a specific resistance of 18.2 MΩ cm.

**Methods.** Following the classical thin film hydration method,19
the effects of NSAIDs in multilamellar liposomes (MLV) of
DPPC were studied by comparing the thermotropic behavior of the
drug homogeneously dissolved with DPPC in an organic solvent
direct mixing procedure) with that observed by leaving a fixed
amount of drug in contact with already-prepared vesicles (incubation
procedure).

**Incubation Procedure.** DPPC was dissolved in a chloroform–
methanol (9:1 v/v) mixture in a round-bottomed flask. The solvents
were removed at 40 °C on a rotary evaporator under a nitrogen
stream until the lipid was dried and distributed as a thin film on the
wall of the vessel. The resulting film was kept under vacuum overnight
to remove the residual solvents. The lipid films were hydrated by
adding 10 mM HEPES buffer to the film and then alternately heated
in a water bath at 60 °C and mixed via a vortex mixer at room
temperature for 5 min. This procedure was repeated three times.
Samples for DSC measurement were then prepared by adding certain
amounts of the concentrated stock solutions of the NSAIDs to the
liposome suspensions in order to obtain the chosen molar fractions
of the compound.

The samples were shaken for 1 h in a water bath at 60 °C, a
temperature above the DPPC gel to liquid-crystalline phase transition,
to allow full hydration of the phospholipid and homogenize the
liposomes, permitting the NSAIDs to reach the partition equilibrium
between the lipid membranes and the aqueous medium. Finally, the
samples were aged overnight at 4 °C and again shaken at room
temperature for 20 min before the measurement.

**Direct Mixing Procedure.** NSAIDs were mixed with DPPC in
organic solvents (9:1 v/v chloroform–methanol) in order to obtain
the required molar fraction of the drug. After the dissolution of
the compounds, the preparation of the samples followed the same steps
described in the incubation procedure: lipid film preparation,

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rehydrated with 10 mM HEPES buffer at a temperature above the gel to liquid-crystalline phase transition alternating with vortex mixing, aging overnight at 4 °C, and shaking at room temperature for 20 min.

DSC measurements were performed using a microcalorimetry system (MCS DSC, MicroCal Inc., Northampton, MA) as described elsewhere. Samples of NSAID-containing multimolecular vesicle suspensions in buffer (~1 mg/mL) and the buffer used as reference were degassed before measurement and then were transferred to the DSC sample holder. During the measurements, the samples were kept under nitrogen overpressure. Each sample was examined in triplicate to check the reproducibility of the results. The scan rate employed was 1 °C/min in the temperature range between 10 and 55 °C after an initial isothermal period of 15 min. The obtained data were analyzed using Microcal Origin software. After the calorimetric scans, aliquots of the scanned samples were used to determine the amount of phospholipid by phosphorus assay.

For X-ray diffraction measurements, dispersions of DPPC were prepared in 10 mM HEPES buffer (15–20 wt % of lipid in buffer) by the direct mixing procedure as described above. For the chosen NSAID/lipid molar ratios, the respective amount of drug was added from a stock methanol solution before the formation of the thin lipid films. After the hydration procedure and formation of lipid vesicles, the samples (30 μL) used to fill glass capillaries (Hilgenberg GmbH, Malsfeld, Germany; wall thickness 0.1 mm), which were flame sealed and stored at 4 °C before the measurements.

Small- and wide-angle scattering data where collected using soft condensed matter beamline A2 at storage ring Doris III of HASYLAB (DESY, Hamburg, Germany). The synchrotron beam is located in the lipid bilayer. In fact, the presence of an additive in phospholipid bilayers affects thermodynamic parameters such as the main transition and the pretransition temperatures (Tm, Tp), the heat capacity of the samples (c), and the transition enthalpies (ΔH). Compounds entering the lipid bilayers can change the lipid packing mode and therefore change both Tm and ΔH of the gel to the liquid-crystalline transition.

Fully hydrated DPPC bilayers show a characteristic thermal transition consisting of a broader low-enthalpy pretransition at 35.3 °C and a sharp high-enthalpy main transition at 41.2 °C. Below 35 °C, pure DPPC bilayers form the lamellar gel phase (Lg), where the acyl chains are in the all-trans conformation and are tilted from the bilayer normal. Above 41 °C, the lamellar liquid-crystalline Lc phase with fluid acyl chains is formed. Between 35 and 42 °C, the phospholipid bilayers form the so-called ripple phase Pcr.

Calorimetric heating curves of DPPC liposomes in HEPES buffer at pH 7.4 in the absence and presence of different molar fractions of NSAIDs indomethacin, acemetacin, and nimesulide are shown in Figure 2. Thermograms of DPPC in buffer are shown in the top row, where the pretransition and main transition temperatures are in good agreement with those previously published.

Depending on the molecular structure and the mole fraction used, the NSAID studied have different influences on the main transition temperature Tm (Figure 3) and on the cooperativity of the phase transition (e.g., full width at half-maximum of the transition peak). Adding nimesulide decreases the Tm values; however, at a relatively high molar fraction of 0.4, only a small reduction of 1.2 °C is found. Arylacetic acid derivatives acemetacin and indomethacin show a larger shift in the transition temperatures, and at a molar fraction of 0.4, a reduction of up to 5 °C (acemetacin) is obtained. These findings suggest that these compounds show a higher affinity toward the lipid membrane and better penetration capabilities. The arylic acid derivatives are chemically very similar, yet acemetacin differs from indomethacin because it contains a different residue in

Indomethacin, and nimesulide at pH 7.4. Lipids and decrease the cooperativity of the phase transitions.33,34

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Figure 2. Calorimetric heating curves of DPPC bilayers (top row) containing different molar fractions \(x_{\text{NSAID}} = 0.9\) (middle row; \(x_{\text{NSAID}} = 0.6\) - bottom row) of the NSAIDs studied [(A) acetaminophen, (B) indomethacin, and (C) nimesulide] at pH 7.4.

Figure 3. Effect of increasing molar fractions of the NSAIDs (○, acetaminophen; ■, indomethacin; ▲, nimesulide) in DPPC/NSAID mixtures on the \(T_m\) values (average of at least three runs). The samples are prepared using the incubation procedure or the direct mixing procedure (filled symbols).

Figure 4. Effect of increasing molar fractions of the NSAIDs (○, acetaminophen; ■, indomethacin; ▲, nimesulide) in DPPC/NSAID mixtures on the \(T_m\) values (average of at least three runs). The samples are prepared using the incubation procedure or the direct mixing procedure (filled symbols).

The enthalpy changes \((\Delta H)\) determined from the peak area remained nearly constant (data not reported because of the negligible variation). This behavior is similar to that observed by the interaction between lipophilic or amphipathic drugs and DPPC liposomes, as also shown previously35–37 and explained in terms of a “fluidizing” effect due to the introduction of lipophilic drug molecules into the ordered structure of the lipid bilayer. The drug molecules can intercalate between the flexible acyl chains of lipid as interstitial impurities, causing \(T_m\) variations without changing \(\Delta H\).38

The shift of the \(T_m\) values obtained by the direct mixing procedure must be considered to be the maximum effect obtainable if the entire drug was able to reach the vesicle surface, penetrate it, and homogeneously diffuse through the lipid layers. Therefore, this shift is much larger compared with the results observed for the preparation by incubation (Figure 4).\(T_m\) shifts resulting from drug incubation are smaller, suggesting that the drug migration through the aqueous medium is initially greatly hindered and that only a small amount that reaches the bilayer surface is able to penetrate the vesicles.

Changes in the phase-transition parameters and the complete absence of the pretransition indicate that changes in the structure of the liquid-crystalline and gel phases of the DPPC dispersion must be taken into account. To study the structural effect of NSAIDs on DPPC bilayers, SANS/WAXS studies have been carried out. Figure 5 shows the SAXS region of DPPC in buffer and in solutions with 40 mol % of the drugs at 20 °C. The DPPC multilayers in buffer are well ordered. The peaks show a small fwhm, indicating a good correlation between the bilayers (Table 1). At 20 °C, the \(L_p\) phase has a \(d\) value of 6.37 nm, which is in good agreement with literature data.39 The addition of 10 mol % nimesulide does not change the \(d\) value in the gel phase but reduces the correlation length between the bilayers drastically (Table 1). A larger amount of nimesulide (mole fraction of 0.4) yields a small increase in the \(d\) value to 6.5 nm and a further decrease in the correlation length. The chain packing is also not significantly influenced by the addition of 10 mol % nimesulide as seen in the WAXS patterns (Figure 6). Two peaks characteristic of an orthorhombic lattice with tilted chains can be resolved at the same positions as for DPPC in buffer (2.36 and 2.41 nm\(^{-1}\)). The increase in the nimesulide concentration to 40 mol % moves the peak positions (2.37 and 2.38 nm\(^{-1}\)), which can be explained

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by a reduced chain tilt that automatically leads to the slightly increased \( d \) value.

The addition of 10 mol % indomethacin leads to the splitting of the first-order Bragg peak observed in the SAXS region (Figure 7). One peak is located at a \( d \) value of 6.97 nm, and the other reflection has a spacing of 7.7 nm. Obviously, there is a phase separation, and the amount of indomethacin is not large enough to reach a homogeneous distribution. If the indomethacin concentration is increased to 40 mol %, then one homogeneous phase with a \( d \) value of 7.6 nm is formed. The chain packing is also drastically influenced because only one Bragg peak has been observed, which indicates a hexagonal packing of non-tilted chains (L\(_{\beta}\) phase). The position of this peak corresponds

![Figure 5](image5.png)

Figure 5. Small-angle X-ray diffraction patterns recorded in static exposures at 20 °C for DPPC (a) and subsequent mixtures with nimesulide (b), indomethacin (c), and acemethacin (d) (\( \chi_{\text{DPPC}} = 0.6 \)).

![Figure 6](image6.png)

Figure 6. Wide-angle X-ray diffraction patterns recorded in static exposures at 20 °C for DPPC (a) and subsequent mixtures with nimesulide (b), indomethacin (c), and acemethacin (d) (\( \chi_{\text{DPPC}} = 0.6 \)).

![Figure 7](image7.png)

Figure 7. Small-angle X-ray diffraction patterns of DPPC mixed with indomethacin [(a) \( \chi_{\text{DPPC}} = 0.9 \), (b) \( \chi_{\text{DPPC}} = 0.6 \)] recorded in static exposures at 20 °C.

![Figure 8](image8.png)

Figure 8. Small-angle X-ray diffraction patterns of DPPC (a) and subsequent mixtures (\( \chi_{\text{DPPC}} = 0.6 \)) with nimesulide (b), indomethacin (c), and acemethacin (d) recorded in static exposures at 50 °C.

![Figure 9](image9.png)

Figure 9. Pressure—area isotherm of DPPC on HEPES buffer (a) and on HEPES buffer containing 1 mM indomethacin (b) at 20 °C.

### Table 1. Bilayer Spacing (\( d \)) and Correlation Length (\( \xi \)) as Calculated from the X-ray Diffraction Analysis for DPPC and the Mixtures Investigated at 20 and 50 °C

<table>
<thead>
<tr>
<th>( \chi_{\text{DPPC}} )</th>
<th>DPPC</th>
<th>DPPC + nimesulide</th>
<th>DPPC + acemethacin</th>
<th>DPPC + indomethacin</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 °C</td>
<td>50 °C</td>
<td>20 °C</td>
<td>50 °C</td>
<td>20 °C</td>
</tr>
<tr>
<td>( d )(( \AA ))</td>
<td>( \xi )(( \AA ))</td>
<td>( d )(( \AA ))</td>
<td>( \xi )(( \AA ))</td>
<td>( d )(( \AA ))</td>
</tr>
<tr>
<td>DPPC</td>
<td>63.7 ± 0.5</td>
<td>1084 ± 10</td>
<td>65.8 ± 0.5</td>
<td>1410 ± 10</td>
</tr>
<tr>
<td>0.9</td>
<td>63.7 ± 0.5</td>
<td>286 ± 10</td>
<td>68.0 ± 0.5</td>
<td>480 ± 10</td>
</tr>
<tr>
<td>0.6</td>
<td>69.7 ± 0.5</td>
<td>467 ± 10</td>
<td>60.6 ± 0.5</td>
<td>706 ± 10</td>
</tr>
<tr>
<td>0.5</td>
<td>77.0 ± 0.5</td>
<td>368 ± 10</td>
<td>67.6 ± 0.5</td>
<td>236 ± 10</td>
</tr>
</tbody>
</table>

The strongest influence on the DPPC structure is observed for acemetacin, with 10 mol % being sufficient to increase the \( d \) value from 6.37 to 7.46 nm. The increase in the acemetacin concentration does not change this value remarkably (7.73 nm) and does not lead to any phase separation. The bilayer correlation...
is again drastically reduced. The increase in the acemetacin concentration reduces the correlation length further. As observed for indomethacin, the chain packing is hexagonal (one Bragg peak at $2.39 \, \text{nm}^{-1}$) with a cross-sectional area of $0.203 \, \text{nm}^2$.

Compared with DPPC in buffer ($L_{\alpha}'$ phase), the $d$ value in the gel phase ($L_{\alpha}$ phase) is increased by 1.1 nm as a result of the interaction with acemetacin. Assuming that the chains are in an all-trans conformation, the change from the tilted ($30^\circ$) to the non-tilted state would increase the $d$ value by about 0.54 nm. The remaining 0.56 nm strongly suggests that the head group conformation and the hydration must also be changed. Obviously, the interaction of acemetacin with the DPPC head groups alters their orientation to a more stretched conformation, but such a conformational change alone cannot explain the large increase in the $d$ value. Therefore, we conclude that the hydration behavior is affected, leading to a thicker water layer between the lipid bilayers.

Figure 8 shows the SAXS region in the $L_{\alpha}$ phase above the main phase-transition temperature. In all cases, the addition of NSAIDs leads to a pronounced reduction of the bilayer correlation.

**Figure 10.** Diffracted intensities summarized over six $Q$ intervals ($\Delta Q = 0.18 \, \text{Å}^{-1}$, starting from 0.0 Å$^{-1}$) as function of $Q_{xy}$ for DPPC on HEPES buffer containing 1 mM indomethacin at 20 °C and different lateral pressure values (19.5, 25, 30, 40, and 20 mN/m. Schematic representation of the interactions of indomethacin with the monolayers at each lateral pressure assayed.

**Figure 11.** Tilt angle of DPPC as function of lateral pressure at 20°C measured on different subphases (●, water; ○, PBS or HEPES; ▲, HEPES containing 1 mM indomethacin).

<table>
<thead>
<tr>
<th>pressure $\pi$ (mN/m)</th>
<th>$Q_{xy}$ (Å$^{-1}$)</th>
<th>$Q_z$ (Å$^{-1}$)</th>
<th>$A_0$ (Å$^2$)</th>
<th>$Q_{xy}$ (Å$^{-1}$)</th>
<th>$Q_z$ (Å$^{-1}$)</th>
<th>$A_0$ (Å$^2$)</th>
<th>tilt $t$ (deg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.5</td>
<td>1.474</td>
<td>21.0</td>
<td></td>
<td>30.3</td>
<td>1.476</td>
<td>20.9</td>
<td>1.344</td>
</tr>
<tr>
<td>25</td>
<td>0.71</td>
<td>0.62</td>
<td>0.08</td>
<td>27.9</td>
<td>0.65</td>
<td>0.57</td>
<td>0.08</td>
</tr>
<tr>
<td>40</td>
<td>0.59</td>
<td>0.49</td>
<td>0.09</td>
<td>1.482</td>
<td>0.59</td>
<td>0.49</td>
<td>0.09</td>
</tr>
<tr>
<td>20</td>
<td>0.68</td>
<td>0.68</td>
<td>0.68</td>
<td>1.473</td>
<td>21.0</td>
<td>1.351</td>
<td>1.466</td>
</tr>
</tbody>
</table>

* Phase I is the strongly influenced DPPC phase with upright oriented molecules, and phase II is the less influenced phase with tilted molecules.

In contrast to the behavior in the gel phase, the addition of 10 mol % nimesulide increases the $d$ value slightly to 6.8 nm. Such an increase could be the result of a change in the hydration behavior of DPPC due to interaction with nimesulide in the disordered liquid-crystalline phase. The ordered gel phase is not influenced by nimesulide, whereas in the more liquid-like $L_{\alpha}$ phase, interactions are observed.

At 40 mol %, acemetacin as well as indomethacin have very similar influences on the $d$ values in the gel phase. In both cases, the head group conformation and hydration are changed, allowing an upright orientation of the chains. However, pronounced differences between these two systems have been observed in the $L_{\alpha}$ phase above the main transition. In the case of acemetacin, chain melting leads to a decrease in the $d$ value by approximately 0.4 nm. The melting alone is expected to reduce the bilayer thickness by 0.8 nm. Therefore, a further increase in the thickness of the water layer between the lipid bilayers has to be assumed. Such changed hydration behavior can be expected by comparing...
the $d$ values of DPPC in the buffer with those of DPPC in the acematin solution. The difference is approximately 0.6 nm. For 10 mol % indomethacin, the splitting of the peaks also occurs in the $L_a$ phase. Chain melting leads to a decrease in the $d$ values by approximately 1 nm for both coexisting phases. This is slightly more than expected for the pure melting process. From this observation, we conclude that the thickness of the water layer between the bilayers is decreased. The same conclusion can be made for 40 mol % indomethacin, where a reduction of the $d$ value by 1.1 nm is observed. The $d$ value in the $L_a$ phase is therefore very similar to that observed for DPPC in buffer. However, there is also the possibility that indomethacin increases the fluidity in the chain region, leading to a further decrease in the $d$ value. However, indomethacin could increase the thickness of the water layer between the bilayers, leading to an increase in the $d$ value. Both effects can compensate for each other, leading to a $d$ value that is similar to that of DPPC in buffer. But the same $d$ value might not be an indication of the same structure.

Figure 9 presents the area—pressure isotherms of DPPC on buffer and on the 1 mM indomethacin solution measured at 20 °C. DPPC on the buffer exhibits the same phase behavior as on water. The lateral pressure starts to increase below a molecular area of approximately 20.2 Å². A phase transition from the disordered liquid-expanded (LE) phase to an ordered condensed (LC) phase occurs at around 5 mN/m. On the 1 mM indomethacin solution, the isotherm is strongly expanded because of the penetration of indomethacin, and the LE-LC phase transition is shifted to higher lateral pressures around 15 mN/m. Above the phase-transition pressure, the molecular area on the indomethacin solution is still much larger than on the pure buffer. Further compression leads to another change in the isotherm slope around 33 mN/m, indicating that most of the penetrated indomethacin is squeezed out. At pressures above 38 mN/m (kink in the isotherm), the molecular area of DPPC on the indomethacin solution is only slightly larger than that measured on the pure buffer.

GIXD measurements show a large difference between the DPPC chain lattice on buffer compared with that on the indomethacin solution (Figure 10). On the buffer as on water, DPPC exhibits an oblique chain lattice with strongly tilted chains.40,41 The tilt angle decreases from 37° at 10 mN/m to 29° at 40 mN/m (Figure 11). The reason for this large tilt angle is the mismatch between the area requirement of the large, strongly hydrated head group and the tightly packed hydrocarbon chains with a cross-sectional area of approximately 20.2 Å². On the indomethacin solution, only one very broad Bragg peak is observed just above the phase transition (Figure 10). The large fwhm indicates a very disturbed chain lattice with a small correlation length. The Bragg rod has its maximum at zero $Q_z$, indicating an upright orientation of the chains. The cross-sectional area amounts to 21 Å², which is much larger than for DPPC on buffer. Therefore, one can assume that the chains are partially disordered. The molecular area measured by GIXD is much smaller than the area determined from the isotherm (42 compared with 74 Å²). This indicates that indomethacin has penetrated into the DPPC monolayer and occupies a certain space at the interface. The penetration leads to a fluidization of the DPPC layer (higher phase-transition pressure and larger molecular areas). Further compression leads to the appearance of additional Bragg peaks around zero $Q_z$ and at higher $Q_z$ values (Figure 10). The fitting and indexing of these peaks give a chain lattice that is similar to that of DPPC on buffer (oblique lattice). The peak positions as well as the cross-sectional areas of the different phases and the tilt angles are presented in Table 2. The second phase, which appears at 25 mN/m, is an influenced DPPC phase because it clearly has a smaller tilt angle that does DPPC on buffer (Figure 11). Compression leads to a partial squeezing out of indomethacin, which is still interacting with the DPPC head groups. This interaction obviously changes the orientation and/or the hydration of the PC head groups, leading to a smaller area occupied by these head groups. The decrease in the mismatch of the area requirements between the different parts of the DPPC molecules allows the reduction of the chain tilt. Therefore, at higher pressures two differently influenced DPPC phases coexist. The intensity of the broad Bragg peak at zero $Q_z$ decreases drastically above the kink in the isotherm (38 mN/m), indicating an almost complete squeezing out of indomethacin and therefore the disappearance of the nontilted phase. An expansion of the monolayer to 20 mN/m increases the intensity of the broad peak at zero $Q_z$, indicating a repenetration of indomethacin into the DPPC monolayer. This repenetration process is obviously kinetically hindered and needs a certain time. Therefore, directly after the monolayer expansion weak peaks belonging to the less influenced DPPC phase can be seen together with the strong and very broad peak at zero $Q_z$.

Conclusions

The sequence of events resulting from cyclooxygenase inhibition does not totally explain the overall gastric toxicity of NSAIDs. Such mechanisms are complex, and the cascade of events leading to mucosal damage can also be related to NSAID topical irritancy. The therapeutic and toxic effects of NSAID are thus strongly influenced by their lipid affinity. Accordingly, the aim of this article is to report results with regard to the structure and thermodynamic aspects of the interactions of NSAIDs with lipid membranes once these interactions have clinical significance in elucidating the effects of NSAIDs on the integrity of the gastric mucosal barrier, or they may be useful in the development of new NSAIDs.

The NSAIDs interact and penetrate the lipid bilayers and induce variations in the temperature associated with the lipid main phase transition. The arylacetic acid derivatives (acemetacin and indomethacin) induce greater modifications, probably because of better interaction and penetration into the lipid structure. The enthalpy changes ($\Delta H$) remain nearly constant. The calorimetric results obtained in the present work agree with the literature,33,34 where it was reported that the $T_m$ and cooperativity of the phase transition of DPPC liposomes were decreased by the presence of indomethacin. This behavior is explained in terms of a fluidizing effect due to the introduction of lipophilic drug molecules into the ordered structure of the lipid bilayer. The drug molecules intercalate between the flexible acyl chains of lipid as interstitial impurities, causing $T_m$ variations without $\Delta H$ changing.38

The NSAIDs studied revealed perturbing effects of the membrane liquid-crystalline phase, as observed both by SAXS and DSC studies. The observed effects of the NSAIDs are in agreement with the fluidizing effect of these drugs, which have been observed by fluorescence measurements of steady-state anisotropy.42

In the lipid gel phase, indomethacin and acemetacin showed the highest destabilizing effects. Because the model used in the current study is made of the same class of phospholipids that line the luminal aspects of the mucus gel layer to provide it with nonwetting properties, the observed destabilizing effects on

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membrane biophysics may be part of the mechanism by which these NSAIDs attenuate the hydrophobic barrier properties of the stomach’s mucus phospholipid gel layer with the consequent increase in the back diffusion of luminal acid into the mucosa and the development of erosions. In comparison, nimesulide, which has a less damaging gastrointestinal profile, showed minimal effects in the lipid gel phase.

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