Effect of pH and Ibuprofen on the Phospholipid Bilayer Bending Modulus

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The lipid bilayer bending modulus, characterized by thermal undulations, is often affected by the presence of membrane active molecules. However, complex interplay between headgroup charges, hydration, and bilayer structural parameters such as bilayer thickness make it difficult to understand the changes in bending modulus. Using neutron spin−echo measurements, the effect of ibuprofen, a model nonsteroidal anti-inflammatory drug, on the bending modulus of phospholipid membranes is studied as a function of pH and temperature. Ibuprofen was found to lower the bending modulus at all pH values. We present molecular insights into the observed effect on membrane dynamics based on molecular dynamics simulations and small-angle neutron scattering based structural perturbations as well as changes in zwitterionic headgroup electrostatics due to pH and addition of ibuprofen.

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used drugs worldwide.[1] They are used for their antipyretic (fever reducing), analgesic (pain reducing), and anti-inflammatory action. Despite significantly different pharmacokinetics (e.g., release profile) among the different NSAIDs, oral consumption of different NSAIDs results in gastrointestinal (GI) toxicity and mild to fatal ulcers. However, the mechanism by which NSAIDs cause GI toxicity is not fully understood and remains an active area of research. Strong preclinical and clinical evidence gathered by Lichtenberger and co-workers indicates (in oral as well as systemic administration) direct interactions of NSAIDs with zwitterionic phospholipids (GI tract lining) as being primarily responsible for GI toxicity.[2]

The focus of this report is to understand the effect of ibuprofen, a model NSAID, on the membrane bending modulus (κ) as a function of pH using the neutron spin echo technique. The bending modulus not only is known to play a key role in the viscoelastic behavior and stability of special types of cells such as erythrocytes[3] but also is a key determinant in a number of processes such as cell division, fusion, shape changes, adhesion, permeability,[4] and even protein folding.[5] From a biophysical point of view, the membrane bending modulus governs the thermal undulations, which further determines both short-range interactions between membranes and substrates[6] as well as long-range interactions as indicated by phenomena such as anomalous swelling of the repeat distance observed in multilamellar systems.[7]

The intercalation of phospholipid bilayers by small molecules or by transmembrane proteins is expected to lead to significant changes in the mechanical properties of the bilayer and is typically quantified by the bilayer bending modulus. However, specific changes in such properties are known to be a complex interplay of headgroup charges, hydration, and bilayer structural parameters such as bilayer thickness.[8−11] A number of studies suggest that the effect of drug molecules on the membrane mechanical properties can be significant and relates to the drug's therapeutic action or side effects.[4,8,12−16] Recently, Zhou and Raphael showed that salicylate which is similar to aspirin, another popular NSAID, can significantly affect the SOPC lipid membrane bending modulus and area compressibility modulus.[4] On the other hand, a number of NSAIDs have been shown to induce vesicle fusion[12] which is again indicative of a drug's effect on membrane mechanical properties. Mingeot-Leclercq and co-workers showed that antibiotic azithromycin can eliminate domains in DPPC−DOPC systems and disrupts giant vesicles made up of DOPC lipid.[13]

Recently, we have examined the structural perturbations in the phospholipid bilayer introduced by NSAIDs such as ibuprofen[17,18] to understand the interactions between such drugs and membranes as well as developing delivery mechanisms for such drugs that might lower the gastrointestinal toxicity.[2] Here, we experimentally examine the effect of pH and ibuprofen, an amphiphilic NSAID,[1] on the bending modulus of a model bilayer membrane of 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC). With changing pH, without addition of any buffer or ions, we anticipate a change in the electrostatic interactions between lipid molecules and between lipid and water molecules. Further, because of the interactions between the ibuprofen and the lipid membrane and the protonation/deprotonation of the ibuprofen with pH, we expect that the incorporation of ibuprofen into the lipid membrane could significantly alter the mechanical properties of the membrane. Moreover, since such lipid−NSAID adducts[2] have been suggested as a safer means of delivery of anti-inflammatory drugs, it is critical to understand the potential impact on the mechanical properties of the membranes and therefore long-term stability of such adducts.

Using neutron spin echo (NSE) on small unilamellar vesicles, we investigated the effect of ibuprofen (at a drug-to-lipid mole ratio of ≈0.31/1) on membrane dynamics as a function of pH, due to both its physiological relevance and pH-dependent charge state of ibuprofen (neutral when pH < pK_a (≈4.6) and anionic

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when pH > pK_a). Supporting molecular insights based on quantities derived from 100 ns long molecular dynamics (MD) simulations and structural studies using small-angle neutron scattering data from an earlier publication[18] are presented. While several studies of lipid membrane mechanics have been conducted using NSE,[6,7] the work described here examines the role of pH and the role of the protonation state of the intercalant on the membrane dynamics.

Materials and Methods

Neutron Spin Echo. ***Materials.*** 1,2-Dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC) was purchased from Avanti Polar Lipids Inc. (Alabaster, AL) and used without further purification. The chemicals ibuprofen, DCl, NaOD, and D_2O were purchased from Sigma Aldrich (St. Louis, MO). All other chemicals were reagent grade.

Sample Preparation. Neutron spin echo experiments were performed using small unilamellar vesicles (SUV) made of DMPC at a weight fraction of 2% with water, and the ibuprofen−DMPC mole ratio was maintained at 0.31/1. SUVs were prepared by extrusion[19] of well-hydrated dispersions of lipid or drug/lipid mixtures in pD adjusted D_2O (pD was adjusted using dilute solutions of NaOD and DCl and without addition of any buffer). Extrusions were performed using a hand-held miniextruder (Avanti Polar Lipids Inc., AL) by passing the solutions through the polycarbonate membrane of 100 nm diameter pores for a total of 29 times. Dispersions of pure DMPC were prepared by first mixing powder lipid in pD adjusted D_2O with vigorous vortexing followed by hydration at 30 °C (well above the DMPC gel−fluid transition of 23 °C) for at least two hours before being extruded. On the other hand, dispersions containing both ibuprofen and DMPC were made by first codissolving them in chloroform and drying under a stream of N_2 to make thin films. These films were further dried in vacuum overnight (at room temperature) to ensure complete removal of chloroform. Deuterated water (pD-adjusted) was added to these dry samples, which were then vortexed and hydrated in the same manner as pure DMPC solutions before being extruded.

Instrumentation. All experiments were performed on the NG5 beamline Neutron Spin Echo spectrometer at the Center for High Resolution Neutron Scattering (CHRNS) in the NIST Center for Neutron Research[20] facility. The wavelengths of the incident neutron beam (λ) used were 6 and 8 Å, with a $\Delta\lambda/\lambda \approx 17.5\%$. A complete inelastic scatterer made of carbon powder was employed as the standard sample for measuring instrumental resolution. Four detector positions were used to cover a q-range from 0.0343 to 0.1690 $Å^{-1}$ and Fourier times of 0.05−40 ns. The raw data were reduced using the DAVE program provided by NCNR.[21]

Molecular Dynamics Simulations. Atomistic MD simulations were performed for pure 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) lipid and ibuprofen−DPPC systems to obtain information on the location of the drug in the bilayer, local water dynamics near the lipid headgroup region, as well as fluctuations in the bilayer thickness as represented by the distance between the center-of-mass of headgroups of two monolayers. To qualitatively represent the ibuprofen charge state below and above the drug pK_a, separate simulations with neutral and anionic forms of ibuprofen were performed and followed the methodology described previously[17] and briefly described below. DPPC was chosen as the model system, as it is one of the most widely used and well-studied systems in MD simulations. Simulations were performed at 323 K well above the gel−fluid phase transition of DPPC (314 K). The common fluid-like nature of the bilayer in simulations and experiments and identical headgroups for DMPC (experiments) and DPPC (simulations) render the information on local water dynamics around the headgroup region as obtained from MD simulations relevant to the interpretation of the NSE experiments. The membrane dynamics (captured by κ values) are expected to be similar for lipid bilayers comprised of homologous chains only varying slightly in chain lengths. For example, two saturated lipids with identical headgroups and differing by one methylene group in their chains have been shown to have the same κ values within the errors of the measurement.[22]

Methodology. All simulations were performed using the GROMACS 3.3.3 package.[23,24] For the initial structure of a lipid bilayer, a well-equilibrated system containing 128 DPPC lipids and 3655 water molecules was used.[25,26] Periodic boundary conditions were applied in all three directions. This system of 128 DPPC lipids is one of the most widely used for MD studies in the literature and provides a reasonable trade-off between the system size, computational expense, and useful information that can be obtained. The lipid molecules were modeled based on Berger et al.[27] and the *ffgmx* as implemented in GROMACS, both based on GROMOS87 with improvements. The water molecules were modeled using the nonpolarizable simple point charge (SPC) model in which the water molecule is considered to have point charges on oxygen and hydrogen atoms with interactive Lennard-Jones potential parameters applied only with respect to oxygen.[28] An ibuprofen molecule was modeled (both coordinates and partial charges) using PRODRG[29] which is based on the GROMOS87 force field and generates partial charges based on the concept of charge groups such that a single molecular group of bonded atoms (e.g., COO) is assigned an integer charge. The united-atom model was used for both lipid and NSAIDs with explicit hydrogen atoms present only in the water model. The bonds between hydrogen and (nonwater) heavy atoms were constrained using SHAKE[30] while using SETTLE for water.[31] A time step of 2 fs was used, and the integration was based on a leapfrog algorithm as implemented in GROMACS. The Lennard-Jones (LJ) potential was switched at 1.0 nm to go smoothly to zero at 1.2 nm. Electrostatic interactions were computed using a Particle-Mesh Ewald (PME) sum[32] with a direct space cutoff at 1.4 nm and fast-Fourier grid space of 0.12 nm with fourth-order interpolation and a tolerance of 1×10^{-5}. To calculate the short-range LJ and the electrostatic interactions, a neighbor list over 1.4 nm was maintained and updated every 50 fs. The trajectory was saved every 10 ps for further analyses. The pressure and temperature were maintained at 1 bar and 323 K using a weak coupling to an external barostat (time constant 1 ps) and thermostat (time constant 0.1 ps), respectively.[33] The simulation box was allowed to vary independently and isotropically in all directions to maintain the pressure, and the bilayer normal was fixed along the z-direction of the box. VMD software[34] was used for visualization and for drug insertion to create initial structures. Three systems were created: namely, one containing pure DPPC and two systems (one each for ibuprofen-neutral (IN) and ibuprofen-charged (IC)) containing 64 ibuprofen molecules in a lipid bilayer made of 128 DPPC molecules. A net charge of 0 and −1 for the neutral and charged drugs was used to represent the ibuprofen below and above its pK_a (≈4.6[35]), respectively. Ibuprofen molecules were placed within the lipid matrix in about eight layers along the bilayer normal with eight molecules in each layer. A lattice configuration as opposed to randomly chosen locations was used for initial drug locations to make sure that there were no clusters

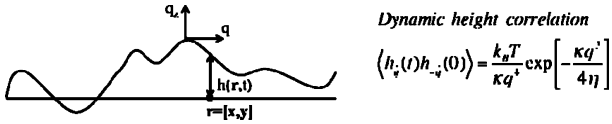

Figure 1. Monge representation of membrane fluctuations in relation to the scattering vector and the dynamic correlation of height in Fourier space (based on Zilman and Granek[37,38]).

in the starting configuration. The total length of the run was 100 ns with the first 40 ns ignored for equilibration. Equilibration was established by observing the system parameters such as potential energy, temperature, pressure, headgroup area, and box volume. Due to the high drug-to-lipid ratio used here, properties such as headgroup area were found to be changing on long time scales and hence the 40 ns equilibration time. All the analyses were done on the last 60 ns of the data. The bilayer was assumed to be symmetric between two monolayers for the purpose of analyses.

Results

The bending dynamic properties of the lipid bilayer membrane as a function of pH and addition of ibuprofen were examined using neutron spin echo (NSE). NSE probes thermally driven stochastic dynamics of membrane interfaces, defined by the height variable $h(x,y)$ in Monge representation,[36] and directly measures the dynamics of height−height correlations via the intermediate structure factor, $S(q,t)$. Zilman and Granek (ZG)[37,38] derived the relation between κ and $S(q,t)$ based on the Helfrich theory.[39] On the basis of Helfrich theory for membrane mechanics, the curvature free energy per unit area of membrane[39] is given as: $f = 0.5\kappa(c_1 + c_2 - 2c_0)^2 + \bar{\kappa}c_1c_2$, where c_0, c_1, and c_2 are the spontaneous and two-principal radii of curvature with $\bar{\kappa}$ being the saddle-splay modulus. For the case of unsupported symmetric bilayers ($c_0 = 0$, e.g., DMPC) subjected to thermal undulations with zero surface tension, $f = 0.5\kappa(c_1+c_2)^2 = 2\kappa H^2$, where $H = 0.5(c_1 + c_2)$ is the mean curvature. Defining $h(x,y)$ as the height of the interface in Monge representation (Figure 1),[36] H becomes

$$H = \frac{(1 + h_x^2)h_{yy} + (1 + h_y^2)h_{xx} - 2h_xh_yh_{xy}}{2\sqrt{(1 + h_x^2 + h_y^2)^3}} \qquad (1)$$

where the subscript indicates partial derivative, for example $h_x = \partial h/\partial x$. For undulations where the membrane surface is close to being flat, i.e., $h_x \approx 1$ and $h_y \approx 1$, the free energy can be written as $f = 0.5\kappa(h_{xx} + h_{yy})^2$, which in Fourier space becomes $f = [\kappa/(2\xi^2)]\sum_q q^4|h_q|^2$. The function h_q is the Fourier transform of the $h(x,y)$, and ξ^2 is the projected area of the membrane. By equipartition theorem, the static correlations of h_q are given by $\langle|h_q|^2\rangle = k_BT/\kappa q^4$. Here, in NSE, dynamic correlations in $h_q(t)$ are of interest as shown in Figure 1 and relate to the intermediate or dynamic structure factor as

$$S(q,t) = \frac{1}{a^4}\int d^2r \int d^2r' e^{i\vec{q}_\parallel(\vec{r}-\vec{r}')} e^{-q_z^2/2\langle[h(\vec{r},t) - h(\vec{r}',0)]^2\rangle} \qquad (2)$$

where a is a molecular length.

Under the conditions of $q_z \gg (k_BT/\kappa)^{1/2}q_\parallel$,[37,38] the intermediate structure factor measured by NSE is given by $S(q,t) = I(q,t)/I(q,0) = Ae^{-(\Gamma t)^{2/3}}$ and shown for one of the data sets at pH ≈ 7.4 in Figure 2a. The decay constant Γ, as derived by Zilman and Granek (ZG),[37,38] for randomly oriented membrane bilayer sheets has a q^3 dependence with the prefactor related to the bending modulus of the bilayer and is given as

$$\Gamma = \left(0.025\gamma\frac{k_BT}{\eta}\right)\left(\frac{k_BT}{\kappa}\right)^{1/2}q^3 \qquad (3)$$

which further leads to

$$S(q,t) = A\exp\left[-\left(0.025\gamma\frac{k_BT}{\eta}\right)^{2/3}\left(\frac{k_BT}{\kappa}\right)^{1/3}q^2t^{2/3}\right] \qquad (4)$$

The factor γ ($= 1 - 3\ln(q\xi)k_BT/4\pi\kappa$) is ≈1 for $\kappa \gg k_BT$ (valid for lipid bilayers), and η is the solvent viscosity experienced by the bilayer and usually taken as $\approx 3\eta_{D2O}$[40,41] to capture the effect of local aqueous environment on the viscous dissipation of membrane undulations. The viscosity values for the D_2O[42] (η_{D2O}) were taken to be 0.979×10^{-3} kg/ms at 30 °C and 0.840×10^{-3} kg/ms at 37 °C. A plot of $\ln[I(q,t)/I(q,0)]$ vs $q^2t^{2/3}$ (master curve) is a straight line (Figure 2b), and it has been used to determine κ. The use of the same local η at all pH values and for the ibuprofen−lipid adducts and other aspects of local dynamics were probed using MD simulations as detailed below. The inferred bending modulus for the lipid bilayer as a function of pH with and without ibuprofen is shown in Figure 2c.

To obtain molecular insights into the effect of pH and ibuprofen on the bilayer bending modulus, it is critical to understand where the drug is located as a function of pH or its charge state. The MD simulations presented here as well as our earlier free energy calculations[17] indicate that ibuprofen is located deeper into the alkyl chains in neutral form ($pH < pK_a$) and near the headgroup region in anionic form ($pH > pK_a$) as shown in Figure 3a. On the other hand, the local dynamics of water close to the lipid headgroup region was probed by calculating the mean-squared displacement (MSD) of water molecules that fall within 5 Å of the center of mass of the group of lipid headgroup atoms (P, N, and O) and is shown in Figure 3b. The similarity of MSD for pure DPPC, ibuprofen (neutral)−DPPC, and ibuprofen (charged)−DPPC suggests that the η_{D2O} close to the lipid bilayer is unaffected by the pH or the presence of ibuprofen and validates the use of the same local η in the equation for the master curve at all pH values and for the ibuprofen−lipid adducts.

Fluctuations in the bilayer thickness were also probed as an additional deformation mode in the systems studied in MD simulations. Bilayer thickness was defined as the distance between the centers of mass of the headgroup of two monolayers. For this purpose, the headgroup is defined as the group containing all the atoms in the lipid headgroup up to the phosphate oxygen. The deviation of the thickness from the average thickness (estimated using data from 40 to 100 ns) was first estimated from which root-mean-square (rms) values for fluctuations were calculated. rms values were found to be ≈0.189 ± 0.002 Å for DPPC, ≈0.174 ± 0.002 Å for ibuprofen (neutral)−DPPC, and ≈0.173 ± 0.002 Å for ibuprofen (charged)−DPPC. Nearly comparable values for rms suggest that perhaps fluctuations in bilayer thickness do not contribute toward the apparent bending modulus differences between pure DPPC and ibuprofen−DPPC systems.[43]

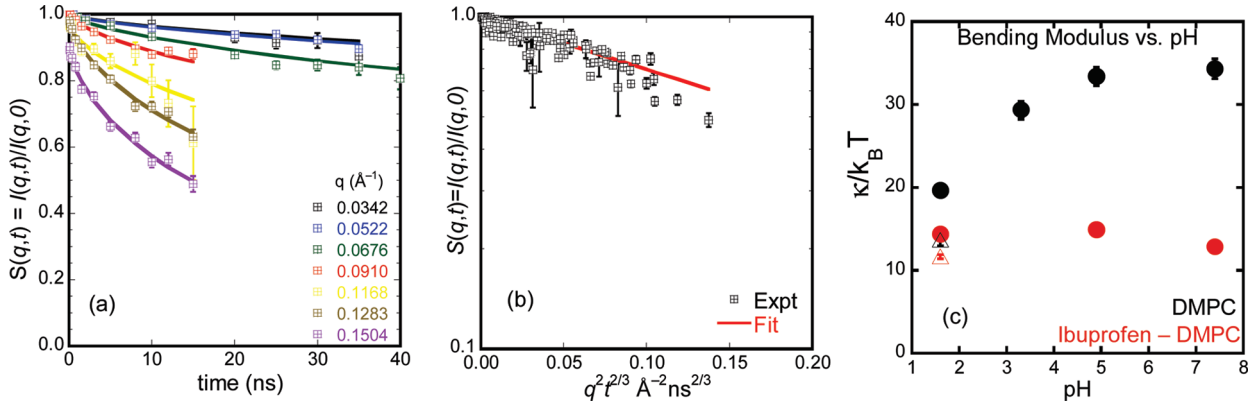

Figure 2. (a) Intermediate structure factor for DMPC at pH ≈ 7.4 and 30 °C. (b) Master curve based on Zilman−Granek scaling (eq 4 in the text). (c) Membrane bending modulus (κ) as a function of pH for DMPC and ibuprofen−DMPC (mole ratio 0.31/1) at 30 °C (solid circles). Also shown are the data for pH ≈ 1.6 and 37 °C (open triangles). The errors in the parameters A and κ were estimated using a weighted fitting to the master curve with the experimental errors as weights.

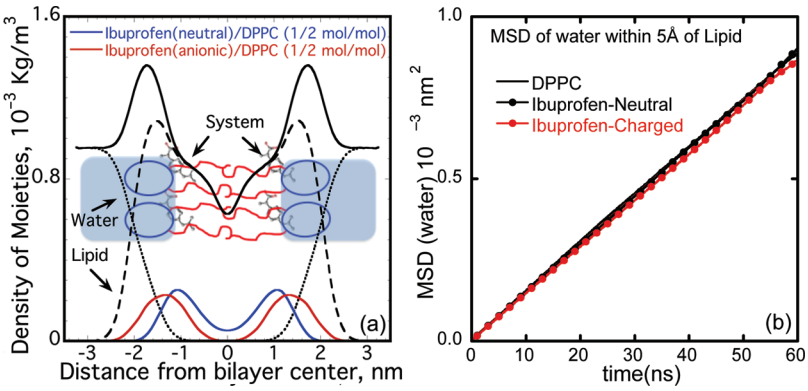

Figure 3. (a) Location of ibuprofen in neutral and anionic forms as predicted by MD simulations. (b) Mean-squared displacement of the water within 5 Å of the lipid headgroup atoms.

Discussion

NSE results show that the value of κ for the pure lipid membranes at 30 °C is relatively unchanged at high pH and significantly decreased at low pH values. On the other hand, with the addition of ibuprofen to the lipid, the κ of the membrane bilayer is unchanged with pH and lower than that of the pure lipid. Moreover, at a pH of ≈2 and at 37 °C, above any gel to fluid transitions for the lipid, the values of κ for the membranes with and without ibuprofen are roughly identical. The inferred values of κ from NSE are a combination of different modes of deformation including bending, area deformation, shear (zero for a bilayer in fluid phase), peristaltic motion from thickness fluctuations, and molecular protrusion.[44] Further, the membrane bending modulus is influenced separately by both the hydrophobic chains and the hydrophilic headgroup.

Since the surface charge density (σ) of the zwitterionic lipid bilayer is expected to change with pH, the contribution due to the electrostatic component (κ_{el}) to κ is expected to change with pH. Zhou and Raphael[4,45] recently addressed the effect of pH on κ_{el} in SOPC and observed its contribution to the total κ change from ≈5% (pH ≈ 2) to ≈40% (pH ≈ 9). This is further bolstered using qualitative estimates of κ_{el} based on the method developed by Winterhalter and Helfrich.[46] Phosphatidylcholine (PC) lipids are known to be positive at low pH, zwitterionic at pH ≈ 4, and negative at high pH values due to interactions between the headgroup moieties (PO_4^- and $N(CH_3)_3^+$) with H^+ and OH^-.[47] Due to the complex charge states of PC and difficulty in estimating σ accurately, a qualitative approach is adopted in estimating the κ_{el}. Winterhalter and Helfrich[46,48] obtained

$$\kappa_{el} = \left(\frac{2k_BT}{e}\right)^2 \lambda\varepsilon_w \frac{Q-1}{Q(Q+1)}\left[Q+2-2\frac{F}{Q+F}\right] \quad (5)$$

where $Q = (1+(\sigma e\lambda/2\varepsilon_w k_B T)^2)^{1/2}$, $F = (\varepsilon_l\lambda/\varepsilon_w h)$, and $\lambda = (\varepsilon_w k_B T/2e^2 n_0)$ are the Debye length with e electronic charge, $\varepsilon_l(\varepsilon_w)$ dielectric constants for lipid (water), $h(=d/2)$ monolayer thickness, and n_0 bulk concentrations of counterions (H^+/OH^-). Estimates of the Debye length, as a function of pH, are 1, 10, 100, and 700 nm for pH of 1, 3, 5, and 7, respectively, and indicate its extreme sensitivity to n_0 and pH. At low pH, the headgroup charges are screened, and the two monolayers are roughly uncoupled at a pH of ≈1. Using the full expression and approximate σ (≈0.5 C/m^2) for pH < 4 leads to $\kappa_{el} \approx k_BT$, and therefore κ_{el} is insignificant. On the other hand, for pH > 4, where the headgroup charges are not screened, using an asymptotic expression $\kappa_{el} = (2k_BT/e)^2(\varepsilon_w/\chi)$ and $Q \gg 1$ leads to values of κ_{el} at least 2 orders of magnitude higher than those at low pH and potentially a significant fraction of the overall κ.

Other sources for the observed changes in κ may emerge from changes in the physical properties of the acyl chains and from possible changes in the area compressibility modulus. From our earlier studies,[18] the membrane gel−fluid transition temperature (T_m) for DMPC is unchanged for pH > 3 but increases by ≈8 to 12 °C for pH values below 2, presumably due to the protonation of the phosphate group ($pK_a \approx 1.5$[49]). Ibuprofen

TABLE 1: Membrane Bending (κ) and Area Compressibility Modulus (K_A) as a Function of pH and at 30 °C (Data in Parentheses Correspond to $T = 37$ °C)

	DMPC		ibuprofen−DMPC (mole ratio 0.31/1)	
pH	κ/k_BT	K_A (mN/m)	κ/k_BT	K_A (mN/m)
1.6	18.0 ± 0.5	163 ± 8	12.2 ± 0.3	153 ± 8
	(13.2 ± 0.3)	(147 ± 8)	(12.0 ± 0.2)	(154 ± 7)
3.3	24.6 ± 0.8	267 ± 15		
4.9	26.8 ± 0.9	291 ± 17	12.8 ± 0.3	161 ± 9
7.4	26.2 ± 0.8	285 ± 16	12.3 ± 0.3	154 ± 8

(in neutral and charged forms) partitions into the membrane and lowers T_m at all pH values by ≈1−2 °C. Structural studies using small angle neutron scattering[18] at $T = 30$ °C showed that at low pH values the lipid headgroup area (A) is decreased and bilayer thickness (d) is slightly increased. The addition of ibuprofen, on the other hand, reduces the value of d at all pH values and does not alter the value of A from that of the fluid-like lipid membrane. This observation is similar to the effect of short-chain alcohols on the SOPC lipid membrane.[10]

The bilayer thickness d impacts κ through $\kappa \approx K_A d^2/c$, where K_A is the area compressibility modulus and $c \approx 12-48$.[22,50] For DMPC, κ decreases by ≈32% with a pH change from 8 to 2 at 30 °C. Further, at a pH of ≈2, κ decreases by ≈18% when the temperature is raised from 30 to 37 °C. This decrease in κ with temperature is due to the lipid chain undergoing gel-like to fluid-like transition as well as d decreasing from ≈4.7 ± 0.1 nm (30 °C) to ≈4.3 ± 0.1 nm (37 °C). Moreover, at low pH, the headgroup hydration is slightly lowered suggesting that the decrease in κ with pH has origins in the perturbations to the headgroup (structural and electrostatic).

The effect of ibuprofen on κ can be understood by considering the structural and hydration changes. Ibuprofen decreases the apparent values of κ by ≈53% at high pH when the drug is anionic and locates in the headgroup/chain interfacial region. On the other hand, ibuprofen leaves κ almost unaffected at low pH when it is neutral and locates deeper in the chains. Using κ obtained from spin echo and $d \approx 4.3$ nm for DMPC (in fluid phase) and ≈4 nm for ibuprofen−DMPC, values of K_A were estimated (Table 1).

For DMPC at low pH and high temperature, the decrease in headgroup hydration and phosphate protonation, as noted above, leads to a headgroup region with low surface charge density and therefore represents a low resistance to undulations as well as changes in local area. In the case of ibuprofen−DMPC, as the drug partitions into already fluid-like chains,[17] the resulting value of K_A is comparable to that of DMPC. Thus, the values of κ_{DMPC} and $\kappa_{ibuprofen-DMPC}$ are similar at pH ≈ 2.

At high pH, the headgroup is well hydrated and zwitterionic for pure DMPC, resulting in higher values of K_A. On the other hand, ibuprofen partitions into the headgroup region leading to a reduction in headgroup hydration. This results in a much smaller value of K_A, nearly half that of DMPC as has been observed in a similar drug−lipid system (salicylate−SOPC)[4,45] as well as in the study on the effect of short-chain alcohols on SOPC,[10] and needs direct experimental confirmation for the ibuprofen−DMPC system.

Using a combination of NSE along with structural studies based on SANS and MD simulations, the changes in the bending modulus for DMPC-based membranes with pH and addition of ibuprofen have been examined. Significantly, we observe that κ decreases by ≈40% going from pH ≈ 8 to pH ≈ 2 and attribute it to changes in lipid interactions and headgroup hydration. Further, ibuprofen lowers κ at all pH values due to a combination of bilayer thinning, reduction in headgroup hydration, and a lower area compression modulus. These studies provide a basis for understanding the role of electrostatics and membrane additives in cellular membranes, and their extension to mixed lipid systems might be facilitated by development of coarse-grained simulation methods that can factor in thickness fluctuations as the structural origin for changes in membrane rigidity, hinted in some of the work presented here.

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Supporting Information Available: Complete set of all NSE data and the extraction of the bending modulus and the error in its determination. This material is available free of charge via the Internet at http://pubs.acs.org.

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