Understanding Detergent Effects on Lipid Membranes: A Model Study of Lysolipids

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ABSTRACT Lysolipids and fatty acids are the natural products formed by the hydrolysis of phospholipids. Lysolipids and fatty acids form micelles in solution and acts as detergents in the presence of lipid membranes. In this study, we investigate the detergent strength of a homologous series of lyso-phosphatidylcholine lipids (LPCs) on 1-palmitoyl-2-oleyl-*sn*-glycerol-3-phosphatidylcholine (POPC) lipid membranes by use of isothermal titration calorimetry and vesicle fluctuation analysis. The membrane partition coefficient (K) and critical micelle concentration (cmc) are determined by isothermal titration calorimetry and found to obey an inverse proportionality relation ($cmc \cdot K \sim 0.05$–0.3). The partition coefficient and critical micelle concentration are used for the analysis of the effect of LPCs on the membrane bending rigidity. The dependency of the bending rigidity on LPC membrane coverage has been analyzed in terms of a phenomenological model based on continuum elastic theory, which yields information about the curvature-inducing properties of the LPC molecule. The results reveal: 1), an increase in the partition coefficient with increasing LPC acyl-chain length; and 2), that the degree of acyl-chain mismatch between LPC and POPC determines the magnitude of the membrane mechanical perturbation per LPC molecule in the membrane. Finally, the three-stage model describing detergent membrane interaction has been extended by a parameter D_{MCI}, which governs the membrane curvature stability in the detergent concentration range below the cmc-value of the LPC molecule.

INTRODUCTION

The effect of detergents and surfactants on fluid interfaces is a well-explored field in colloidal chemistry. A classical experiment is to demonstrate the dramatic decrease of the surface tension, γ, of an aqueous interface in the presence of detergents described by the Gibbs adsorption relation. For a freely suspended lipid membrane, the interfacial tension is vanishing, and the mesoscopic and macroscopic conformational properties are determined by the bending elasticity governed by the Helfrich (1) energy functional:

$$\mathcal{H}_{\text{bend}} = \gamma A + \frac{\kappa}{2}\int_{A} dA(2H - 2H_0)^2. \qquad (1)$$

The membrane is characterized by the area, A, the mean curvature, H, and the resistance to bending is governed by the bending rigidity, κ. The spontaneous curvature, H_0, reflects the preferred mean curvature of the membrane, caused by asymmetry arising from 1), differences in the lipid composition of the two membrane leaflets; or 2), differences in solvent composition to which the two membrane leaflets are exposed. The main distinction between the amphiphile constituting the membrane and the detergent is that the latter can partition into both the membrane and the solvent. The effect of detergentlike molecules on κ has only been subjected to minor investigations, e.g., the emulsifying effect of cosurfactants in microemulsions (2). However, it is well established that lipid bilayer membranes are mechanically destabilized by the presence of detergents (3,4). A classical model of surfactant destabilization of lipid membranes is the three-stage model (5), in which 1), surfactants partition into the membrane at low concentrations; 2), mixed micelles coexist with bilayer membranes enriched in detergent above a threshold concentration; and 3), above a second threshold in the detergent concentration, only micelles persist. A more quantitative extension of the three-stage model is based on the partitioning properties of monomeric surfactants in membranes and micelles (6), where the first threshold composition of the membrane (defining the onset of membrane solubilization) can be approximated by the product of the membrane partition coefficient, K, and the critical micelle concentration, cmc. In this study, we show that although these models are able to describe important properties of surfactant lipid systems, they cannot account for the capacity of surfactants to destabilize membranes mechanically. An increased understanding of the interaction between detergentlike compounds and membranes has many applications in membrane biophysics, e.g., the effect of bile salts (7) and antimicrobial peptides (8) on biomembranes, isolation of membrane proteins (9), identification of insoluble membrane fragments (rafts) (10) and furthermore in the development of tumor target drug delivery systems (11). So far, few studies have been focused on the partitioning of lysolipids into membranes (12). Lysolipids have been shown to increase the ion permeability (13), cause changes in the bilayer hydration properties (14), modify membrane channel function (15), and reduce the lysis tension (16).

Submitted September 9, 2009, and accepted for publication January 15, 2010.

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Editor: Reinhard Lipowsky.

0006-3495/10/05/2199/7 $2.00

doi: 10.1016/j.bpj.2010.01.037

In this study, we investigate the surfactant properties of a homologous series of Lyso-phosphatidylcholine lipids (LPCX, where $X = 12, 14, 16$ represents the number of hydrocarbons along the saturated acyl-chain) on 1-palmitoyl-2-oleyl-*sn*-glycero-3-phosphatidylcholine (POPC) membranes. The interaction of LPCs and POPC lipid membranes is quantified by isothermal titration calorimetry (ITC) and vesicle fluctuation analysis (VFA) to determine the partition coefficient (K) and the membrane bending rigidity (κ), respectively. The critical micelle concentration of each lysolipid was, in addition, determined by ITC. The study is conducted in the dilute, excess water regime at 25°C, which is far above the main phase transition of POPC. The information obtained from the membrane partitioning and destabilizing capacity of the LPCs allows us to make a detailed analysis of the effect of the LPCs on the membrane stability. The results are interpreted in terms of a phenomenological model, and we propose a simple criterion for the membrane destabilizing potency of a surfactant.

MATERIALS AND METHODS

Experimental

Materials

1-Palmitoyl-2-oleyl-*sn*-glycerol-3-phosphatidylcholine (POPC; purity >98%), 1-dodecanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (LPC12), 1-tetradecanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (LPC14), and 1-hexadecanoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (LPC16) were obtained from Avanti Polar Lipids (Alabaster, AL). Organic solvents and sugars were obtained from Sigma-Aldrich (St. Louis, MO). All materials were used without further purification.

Giant unilamellar vesicle preparation

Twenty microliters of POPC lipid in chloroform (0.2 mM) was deposited on platinum wire electrodes using a Hamilton syringe. The solvent was subsequently evaporated overnight in a vacuum chamber. Giant unilamellar vesicles (GUVs) were formed by electroformation (17,18) in a 75 mOsm sucrose solution containing LPCs at the desired concentration. All GUV preparations were conducted at 25°C. The vesicles were then resuspended in a 75 mOsm glucose solution containing LPCs at the desired concentration and subsequently thermostated in an observation chamber. Solution osmolarities were regulated using a freezing-point osmometer (Model 3D3; Advanced Instruments, Norwood, MA) and MilliQ water was used throughout the preparation (Millipore, Bedford, MA).

Large unilamellar vesicle preparation

One-hundred-nanometer large unilamellar vesicles (LUVs) were prepared from POPC lipid films, which were rehydrated in a 75 mOsm glucose solution for 60 min followed by extrusion with an Avanti Polar Lipids mini-extruder. The size distribution of the LUVs was checked by dynamic light scattering (Zetasizer Nano; Malvern Instruments, Malvern, UK) and the effective lipid concentration was determined by ICP-AES (Vista AX; Varian, Palo Alto, CA).

Data analysis

Isothermal titration calorimetry

Isothermal titration calorimetry (ITC) measurements were performed on an iTC200 (Microcal, Northampton, MA) with a cell volume of 204 μL. Small aliquots of 100 nm POPC LUVs were injected into a 75 mM glucose solution containing LPC. Data analysis was performed using custom-made software, which includes an improved baseline estimation and fitting of the partition model described below to the data by χ^2-minimization. The baseline is determined by linear regression of the heat transfer data from the last 20% of the time interval between two injections. The obtained regression lines are then interpolated by line segments. The measured standard deviation of data from the regression line is used to estimate the errors σ_i on the integrated heat transfer ΔQ_i for each injection. In Fig. 1 *a*, the full heat transfer curve is shown.

The data is interpreted in terms of a simple partition model,

$$\frac{C_p}{C_L} = KC_f, \quad K = \frac{1}{C_w}\exp\left(-\Delta G_w^{mem}/RT\right), \qquad (2)$$

where C_p is the concentration of lysolipids partitioned into the membrane, C_L is the total lipid concentration, C_f is the free lysolipid concentration in bulk solution, K is the partition coefficient, $C_w = 55.5$ M is the molar concentration of water, and ΔG_w^{mem} is the free energy of partitioning. The amount of lysolipid is conserved during the titration experiment and is given by the ITC cell concentration $C_0 = C_p + C_f$. Enforcing this constraint on the lysolipid

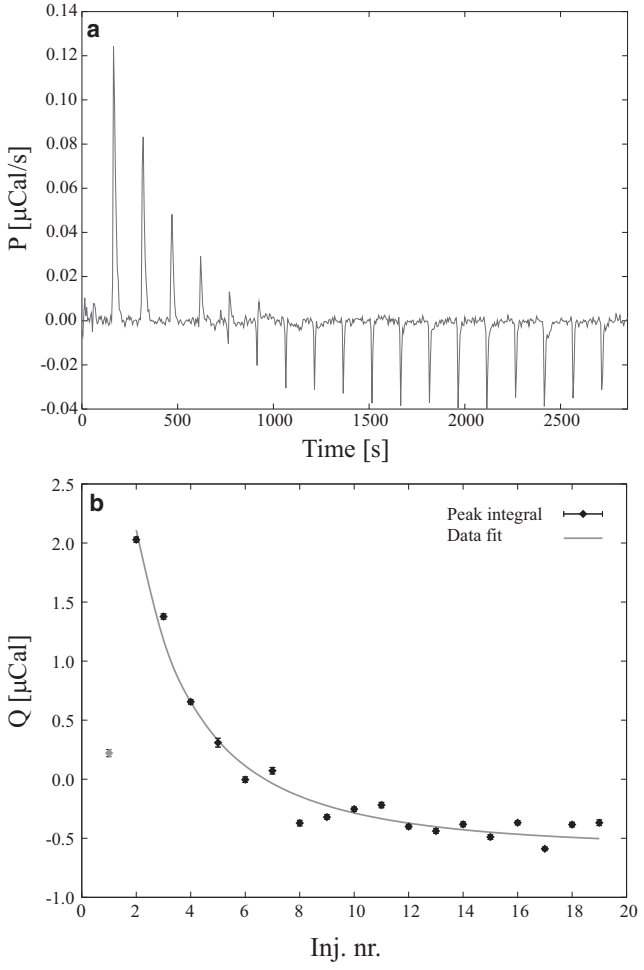

FIGURE 1 Isothermal titration calorimetry injecting POPC LUVs into LPC12 at 25°C. (*a*) Heat-spikes from 2 μL 65.4 mM POPC LUVs injected into a 200 μM LPC12 solution containing 75-mM glucose. (*b*) Peak integrals of the heat-spikes shown in panel *a* as a function of injection number. The peak integrals are fitted using Eqs. 3–5 yielding the partition coefficient, K, the molar enthalpy of partitioning, ΔH_w^{mem}, and the heat of dilution, q_{dil}, as fitting parameters.

concentration, in combination with Eq. 2, yields an expression for the amount of partitioned lysolipid $C_p = KC_LC_0/(1 + KC_L)$. According to Heerklotz and Seelig (6), the cumulant heat, Q_i, is assumed proportional to the amount of compound partitioned into the membrane plus a contribution from heat of dilution. The heat of i^{th} injection is thus modeled as

$$\begin{aligned}\Delta Q_i &= \Delta H_w^{mem} V_{cell} \Delta C_p^i + q_{dil},\\ \Delta C_p^i &= \frac{KC_L^i C_0^i}{1 + KC_L^i} - \frac{KC_L^{i-1} C_0^{i-1}}{1 + KC_L^{i-1}},\end{aligned} \tag{3}$$

where C_0^i and C_L^i are the lysolipid and lipid concentration in the cell after the i^{th} injection, respectively, V_{cell} is the ITC cell volume, ΔH_w^{mem} is the molar enthalpy of partitioning, and q_{dil} is the heat of dilution, which is assumed to be constant. According to Tellinghuisen (19), if instant mixing upon injection is assumed, the lysolipid and lipid concentrations are given by

$$\begin{aligned}C_0^i &= C_0\left(1 - \exp\left(\frac{-V_{add}^i}{V_{cell}}\right)\right),\\ C_L^i &= C_L \exp\left(\frac{-V_{add}^i}{V_{cell}}\right),\end{aligned} \tag{4}$$

where

$$V_{add}^i = \sum_0^i V_i$$

is the cumulant volume injected into the cell upon the i^{th} injection. As C_0, C_L, V_{cell}, and V_i are given by the experimental setup, the remaining unknown parameters K, ΔH_w^{mem}, and q_{dil}, are determined as fitting variables by minimization of

$$\chi^2\left(K, \Delta H_w^{mem}, q_{dil}\right) = \sum_i \left(\frac{\Delta Q_i^{exp} - \Delta Q_i}{\sigma_i}\right)^2, \tag{5}$$

where ΔQ_i^{exp} is the experimentally measured heat from each injection (shown in Fig. 2 *b*) and ΔQ_i is given by Eq. 3. The experimental error associated with the heat of injection, σ_i, is estimated from the ITC thermogram as described previously. The results are compiled in Table 1 and are averages of 3–6 independent ITC experiments.

Bending rigidity measurements

Large unilamellar vesicles were cultivated by electroformation of POPC films hydrated in a 75 mM sucrose solution containing LPC. Suspending the vesicles in a 75 mM glucose solution ensures both improved phase contrast and sedimentation of the GUVs at the bottom of the observation chamber. Preparation of GUVs with the partitioning agent present in the hydration solution ensures that 1), the LPCs are distributed evenly between the two monolayer leaflets of the bilayer; and 2), the lysolipid reservoir is large and the concentration can therefore be considered as constant. The free (bulk) concentration of lysolipid in the GUV sample can, as a consequence, be estimated by the LPC concentration in the hydration solution because only a small fraction of lysolipid has partitioned into the lipid membrane ($K \simeq 10^2 - 10^4\ M^{-1}$ and $C_L^{GUV} \simeq 10^{-7}$ M, which results in $C_p/C_0 \simeq KC_L < 10^{-3}$). In a temperature-controlled chamber (25°C), undulating vesicles are visualized using phase contrast microscopy (Axiovert S100; Carl Zeiss, Oberkochen, Germany). The chamber is sealed to prevent evaporation from the solution and possible changes of concentrations during the experiment. For each measurement, a set of 4000–6000 GUV contours is collected as described in earlier work (20). Each estimate of the bending rigidity is an average obtained from measurements of ~20 individual vesicles. The data analysis for determination of κ is performed by custom-made software (20). In general, the VFA technique is well suited for measuring the bending rigidity of membranes perturbed by partitioning agents, because the measuring principle is noninvasive and the measuring chamber prevents evaporation.

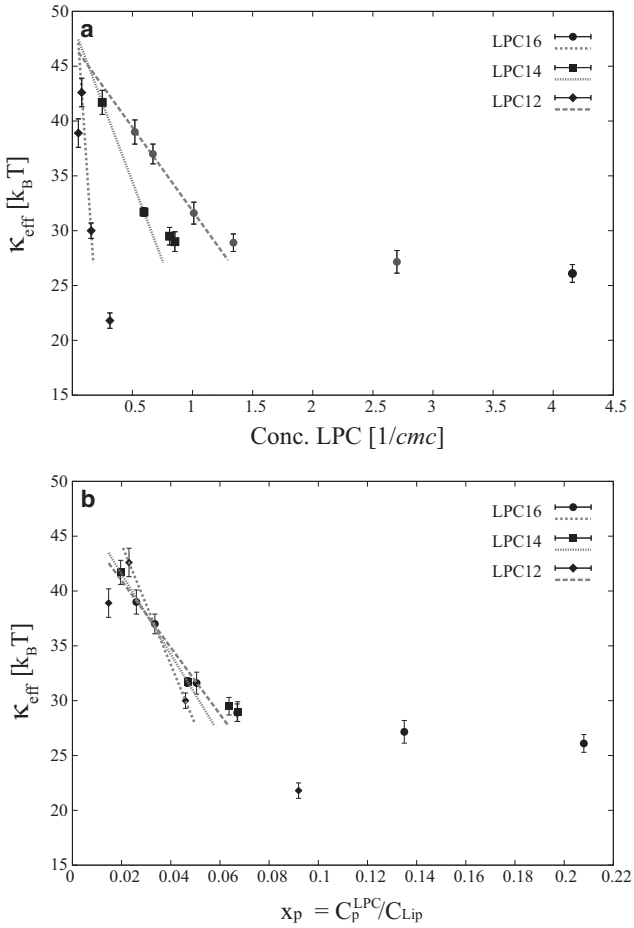

FIGURE 2 Plot of the effective bending rigidity as a function of (*a*) the LPC bulk concentration given in units of the LPC *cmc*-value and (*b*) the LPC membrane molar fraction, x_p, given by $x_p \simeq C_p^{LPC}/C_{Lip} = KC_{LPC}$ for $C_{LPC} < cmc$. Lines representing the fit of Eq. 7 are shown in panel *a* and guidelines emphasizing the differences in LPC influence on κ_{eff} as a function of x_b are shown in panel *b*.

RESULTS AND DISCUSSION

ITC

The titration of POPC LUVs into LPC solutions were all performed at LPC concentrations at which demicellization is

TABLE 1 Parameter values derived from ITC and VFA at 25° C

	LPC12	LPC14	LPC16
K [M^{-1}]	460 ± 50	1750 ± 94	12500 ± 3900
ΔG_w^{mem}[kJ/mol]	−25.1 ± 0.3	−28.1 ± 0.1	−33.3 ± 0.7
ΔH_w^{mem}[kJ/mol]	1.3 ± 0.1	−3.0 ± 0.1	−3.0 ± 0.5
cmc [μM]	640 ± 10	45 ± 2	4*
$K \cdot cmc$	0.29 ± 0.03	0.080 ± 0.006	0.050 ± 0.003
D_{MCI}	2.9	0.6	0.3
λ[Å]	1.8	1.6	1.5

Compilation of the thermodynamic data obtained by ITC at 25°C and mechanical stability parameters assessed by VFA.
*The *cmc*-value of LPC16 was adopted from the literature (12,21), which we verified by fluorescence anisotropy measurements.

neglectable, and partitioning of monomeric LPC into the membrane is thus assumed to be the main contribution of the heat transfer. The *cmc*-values for LPC12 and LPC14 were determined by ITC (data not shown) and the *cmc* of LPC16 was adopted from literature (12,21). Fig. 1 *a* shows heat-spikes from injecting POPC LUVs into LPC12. The heat per injections and the best fit of Eq. 5 to the data is given in Fig. 1 *b*. The obtained values for the partition coefficients and molar enthalpies of partitioning of LPCs into the membrane are given in Table 1. The partition coefficient increases strongly with LPC acyl-chain length with an approximative linear relationship between the free energy of partitioning and the chain length,

$$\Delta G_{\mathrm{w}}^{\mathrm{mem}}/RT = -\ln(55.5\ \mathrm{M}\cdot K) \simeq -0.14 - 0.83X,$$

in agreement with earlier reports (12). The free energy of LPC monomer partitioning into micelles similarly exhibits a linear relationship with X,

$$\Delta G_{\mathrm{w}}^{\mathrm{mic}}/RT = -\ln\,(55.5\ \mathrm{M}/cmc) \simeq 3.83 - 1.27X.$$

For the three different LPCs, $K\cdot cmc \sim 0.05 - 0.3$, showing that the free energy of transferring a monomer from micelle to membrane ($\Delta G_{\mathrm{mic}}^{\mathrm{mem}} = -RT\ \ln(K\cdot cmc) > 0$) is positive and that the process does not occur spontaneously. In work of Heerklotz and Seelig (6), the product, $K\cdot cmc$, was suggested as a measure of a surfactant's capacity to solubilize a membrane. In their classification of surfactants, $K\cdot cmc \leq 1$ ($\Delta G_{\mathrm{mic}}^{\mathrm{mem}} \geq 0$) infers a strong detergent whereas $K\cdot cmc \geq 1$ ($\Delta G_{\mathrm{mic}}^{\mathrm{mem}} \leq 0$) is a weak detergent. By this definition of detergent strength, the LPCs investigated in this study are all strong detergents and follow the sequence: LPC16 > LPC14 > LPC12.

Bending rigidity

The measurements of membrane bending rigidity were performed over a wide range of LPC concentrations at 25°C. In the case of LPC16, GUVs could be studied at concentrations ranging from zero to values above the *cmc* value of LPC16. For LPC12, GUV formation was only possible in a limited concentration-range below *cmc*, whereas for studies of LPC14 the GUVs lost their optical contrast near the *cmc*-value of LPC14. The overall trend is a dramatic decrease in the observed bending rigidity with increasing LPC concentrations, except at the lowest LPC concentrations where minor increases in the bending rigidity are observed (Fig. 2).

In Fig. 2 the bending rigidity is plotted as a function of 1), the bulk/free LPC concentration scaled with respect to the LPC *cmc*-value; and 2), as a function of the LPC membrane molar fraction $x_{\mathrm{p}} \simeq C_{\mathrm{p}}/C_{\mathrm{lip}}$. The magnitude of the LPCs' effect on the membrane bending rigidity depends on whether it is measured relative to the bulk or membrane concentration which is evident in Fig. 2. The decrease in κ, when measured as a function of x_{p} or the *cmc*-scaled bulk concentration, is more pronounced as the acyl-chain length of the lysolipids is reduced. Despite the larger partition coefficient of LPC16, the effect on κ per LPC molecule in the membrane is less than for its shorter homologs. For LPC16, a saturation of κ is observed at high concentrations (Fig. 2 *a*), which correlates with the formation of micelles above the *cmc*-value. Above *cmc*, the LPC16 monomer concentration in the solution is approximately constant and no further partitioning of LPC monomers into the membrane occurs. Below *cmc*, the monomer concentration in the solution is well approximated by total lysolipid concentration. From Fig. 2 *b*, it is evident that the LPC molecules reduce the effective bending rigidity in a common range of x_{p}; however, there are differences in the degree of the perturbation caused by the individual LPC molecules depending on acyl-chain length. This effect is evident by the change in κ_{eff} as a function of x_{p}, as indicated by the slope of the guidelines shown in Fig. 2 *b*.

Phenomenological model

The dramatic reduction in the effective bending rigidity at low lysolipid concentration can be interpreted in terms of a simple phenomenological model, which includes the local effect of the LPCs on the membrane mean curvature. The Helfrich (1) free energy (see also Eq. 1), for the case $H_0 = 0$, is extended by adding a free energy contribution arising from the lateral distribution of lysolipids,

$$\begin{aligned}\mathcal{H}_{\mathrm{int}} &= \kappa_0\lambda\int dA(\rho_+ - \rho_-)2H \\ &\quad + k_{\mathrm{B}}T\int dA\big(\rho_- ln\big(a_{\mathrm{lyso}}\rho_-\big) + \rho_+\ln\big(a_{\mathrm{lyso}}\rho_+\big)\big),\end{aligned} \tag{6}$$

where $\rho_\pm$ is the local lateral density of lysolipids in the upper and lower monolayer leaflets and a_{lyso} is the cross-sectional area per lysolipid in the membrane. The bending rigidity of the detergent free lipid membrane is given by κ_0. In a first approximation, a_{lyso} can be set equal to $a_{\mathrm{lipid}} = 70$ Å^2 (22). The second term in Eq. 6 represents the gas approximation of the free energy for the lateral distribution of lysolipids in the membrane monolayers, while the first term models the coupling between the local lysolipid density difference $(\rho_+ - \rho_-)$ and the mean curvature (H). The coupling strength of $(\rho_+ - \rho_-)$ and H is governed by the parameter λ, which has dimension of length. The term $\lambda/2\ (\rho_+ - \rho_-)$ can be identified as the local spontaneous curvature. Stability analysis of the total free energy, $\mathcal{H} = \mathcal{H}_{\mathrm{int}} + \mathcal{H}_{\mathrm{int}}$, show that the coupling term $(\mathcal{H}_{\mathrm{int}})$ in general leads to a reduction of the effective bending rigidity of the membrane (23,24),

$$\frac{\kappa_{\mathrm{eff}} - \kappa_0}{\kappa_0} = -4\lambda^2\rho_0\frac{\kappa_0}{k_{\mathrm{B}}T} = -\frac{4\lambda^2}{a_{\mathrm{lipid}}}\frac{\kappa_0}{k_{\mathrm{B}}T}(Kcmc)\frac{c_{\mathrm{f}}}{cmc}, \tag{7}$$

where ρ_0 is the average lateral density of lysolipid in the membrane. A fit of Eq. 7 to the data shown in Fig. 2 *a* provides an estimate of λ for each of the three lysolipids, which are given in Table 1. The data shown in Fig. 2 and the predictions of the phenomenological model (Eq. 7),

$$\kappa_{\text{eff}} \rightarrow 0 \quad \text{for} \quad \frac{4\lambda^2}{a_{\text{lipid}}}\frac{\kappa_0}{k_{\text{B}}T}(Kcmc)\frac{c_{\text{f}}}{cmc} > 1,$$

suggest an extension of Heerklotz and Seelig's (6) measure of detergent potency by introducing a membrane curvature instability (MCI) parameter:

$$D_{\text{MCI}} = \frac{4\lambda^2}{a_{\text{lipid}}}\frac{\kappa_0}{k_{\text{B}}T}(Kcmc). \tag{8}$$

In this study, the fit of Eq. 7 to the data shown in Fig. 2 *a* yield equal values of κ_0 for all three LPCs, while $K{\cdot}cmc$ and λ decreases with the LPC acyl-chain length leading to significant changes in D_{MCI} (see Table 1). In general, both the extent of partitioning and the local membrane perturbing effect is important for the overall mechanical stability of the membrane subject to the partitioning agents. Both of these effects are incorporated into the parameter D_{MCI}. Within the framework of the presented phenomenological model, the detergent strength is thus defined as strong when $D_{\text{MCI}} > 1$ and weak for $D_{\text{MCI}} < 1$. The value $D_{\text{MCI}} < 1$ corresponds to the classical model of membrane destabilization (5). According to this, micelles and membranes may coexist at concentration levels above *cmc*, and even the smallest lipid-uptake by the micelles will eventually lead to the disappearance of the bilayer membranes with increasing surfactant concentration. In the case $D_{\text{MCI}} < 1$, the phase-line separating the region of mixed micelles and of intact membranes is given by $C_{\text{D}}^{\text{sat}} = (1 + KC_{\text{L}})cmc$, where $C_{\text{D}}^{\text{sat}}$ is the detergent concentration at which the membrane is saturated (6,25). For the case $D_{\text{MCI}} > 1$, the vesicles collapse at a concentration $C_{\text{D}}^{\text{MCI}}$ below *cmc* of the surfactant due to membrane curvature stress. Above this concentration, lipid-surfactant aggregates are characterized by a higher curvature ($1/R$) and a considerable increase in the partition coefficient of $\sim\exp(\kappa/k_{\text{B}}T\ \lambda/R\ \rho a_{\text{lyso}})$ is expected (due to the first term of Eq. 6). The structure of these lipid-surfactant aggregates will depend on the particular system, but tubular membranes of high curvature or tubular micelles are obvious candidates and have been observed in some lipid surfactant systems (26). Furthermore, for $D_{\text{MCI}} > 1$, the phase-line separating the region of intact membranes and the collapsed state is given by $C_{\text{D}}^{\text{MCI}} = (1 + KC_{\text{L}})cmc/D_{\text{MCI}}$, where $C_{\text{D}}^{\text{MCI}}$ is the detergent concentration at which the membrane collapses. The parameter D_{MCI} can be determined via the previous equation if $K{\cdot}cmc$ and the phase line has been determined experimentally, e.g., by ITC. According to this definition of detergent strength, only LPC12 is a strong detergent and the LPCs investigated follow the sequence: LPC12 > LPC14 > LPC16.

Interpretation of λ

The incorporation of LPCs into lipid bilayers is expected to organize with their polar PC-headgroups close to the bilayer interface and their hydrocarbon chain buried into the hydrophobic core of the membrane, as shown in Fig. 3. A single lysolipid in one of the monolayers will perturb the packing properties of the surrounding lipids in a radius characterized by the lateral correlation length ξ (27). For temperatures well above the main phase transition of the lipid membrane, this correlation length is short, at $\xi \simeq$ 1–2 nm. A possible calculation procedure is to consider a membrane square patch (length of 2ξ with zero tension) with a lysolipid incorporated into one of the monolayers and calculate λ by (28)

$$\kappa_0\frac{\lambda}{a_{\text{lyso}}} = \int_{-\delta}^{\delta}\Pi(z)z\,dz, \tag{9}$$

where $\Pi(z)$ is the lateral pressure profile through the membrane of thickness 2δ. This can be done by self-consistent field theory (29,30), molecular dynamics, or coarse-grained simulations of the membrane.

A simpler approach is to make a rough estimate of λ from dimensional analysis by assuming that the lateral effects from a local membrane perturbation, e.g., insertion of a lysolipid, vanish over the length ξ. A simple packing consideration leads to

$$\lambda \simeq \frac{a_{\text{lyso}}}{l}\left(1 + \frac{\Delta n}{\overline{n}_{\text{Lip}}}\right)\frac{1}{N_\xi}, \tag{10}$$

where $\overline{n}_{\text{Lip}} = (n_{sn1} + n_{sn2})/2$ is the average lipid hydrocarbon chain length and $\Delta n = \overline{n}_{\text{Lip}} - X$ is a measure of the chain length difference between lipid and lysolipid. Here $2l$ corresponds to the hydrophobic thickness of the lipid bilayer and N_ξ is the number of lipids within a lateral coherence patch. Assuming $a_{\text{lyso}} = 70$ Å^2, $l_{\text{max}} = 1$ nm, $\overline{n}_{\text{Lip}} = 17$, and $N_\xi = 7$ leads to λ(LPC16) = 1.1 Å, λ(LPC14) = 1.2 Å,

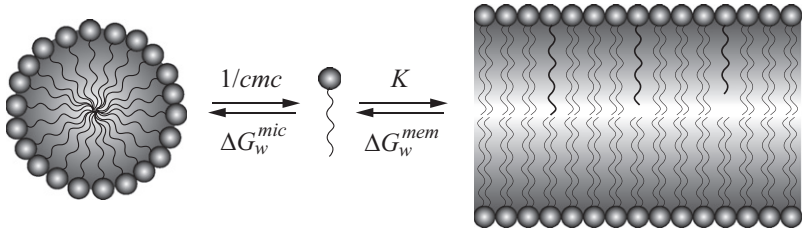

FIGURE 3 Illustration of the LPC equilibria in the presence of a lipid membrane. The partitioning of LPC is given by the partition coefficient, K, and the free energy of partitioning, $\Delta G_{\text{w}}^{\text{mem}}$. The micelle formation equilibrium is described by the *cmc*-value and the free energy of transferring a detergent monomer from bulk to micelle, $\Delta G_{\text{w}}^{\text{mic}}$. The incorporation of LPC molecules in the membrane bilayer is illustrated, showing increased degree of acyl-chain mismatch going from LPC16 to LPC12.

and λ(LPC12) = 1.3 Å, which is a reasonable estimate of the size and trend of λ when compared to the values in Table 1.

Comparison with antimicrobial peptides

The above results have strong similarity with findings on the membrane perturbation of antimicrobial peptides. Antimicrobial peptides are typically small helical peptides that partition into the bilayer leaflets and perturb the membrane by inducing curvature stress. Membrane softening and destabilizing behavior has also been observed with this class of peptides. Gramicidin (31) and Magainin (20,23) are specific examples where a saturation in the bending rigidity is observed with increasing bulk concentration. However, there are no indications of aggregation in the bulk solution to explain this behavior and interaction and aggregation of peptides in the membrane must thus be taken into account. For Gramicidin, the *trans*-membrane dimerization, and for Magainin, the transient membrane pore formation, serve to couple peptides between the monolayers. Consequently, the membrane destabilization parameter, D_{MCI}, must be modified so that *cmc* is replaced by the bulk concentration at which some aggregation of peptides in the membrane takes place. It is interesting to note that the lytic activity of amphiphatic peptides has been observed to increase with both the peptide partitioning into the membrane and the hydrophobic moment, a measure of the capability of a peptide to perturb a bilayer interface (32). Indications of bilayer softening in the presence of cationic surfactants can be inferred from observations of extensive swelling of the lamella phase (33,34). Because high salt concentration (100 mM NaCl) excludes long-ranged electrostatic repulsion as a possible mechanism, the Helfrich (1) steric entropic repulsion due to reduced membrane bending rigidity is the most plausible explanation. Furthermore, deuterium nuclear-magnetic-resonance studies (35) suggest that lipid bilayers are softened by a range of antimicrobial peptides.

CONCLUSIONS

In this article, the interaction of LPC molecules with POPC lipid membranes was investigated using ITC and VFA techniques. The obtained results for the membrane partition properties of the LPCs and the influence on the membrane bending rigidity revealed significant differences in the action of LPCs on the membrane, depending on the LPC acyl-chain length. The longest acyl-chain LPC16 (displaying the smallest acyl-chain mismatch to POPC) gave the weakest perturbation per LPC molecule associated with the membrane, while the LPC12, with the shortest acyl-chain (displaying the largest acyl-chain mismatch) displayed the largest perturbation. Furthermore, GUVs were formed well above the *cmc* of LPC16, whereas studies of LPC12 were only possible at concentrations $C_{\mathrm{D}}^{\mathrm{MCI}} < 0.4 \cdot cmc$(LPC14)— i.e., well below the *cmc*-value. These results point to the conclusion that some detergents are strong in the sense that they mechanically destabilize the membrane through curvature stress, resulting in curvature-induced instability of the GUV, and others are strong detergents in the sense that they form micelles in which the membrane can dissolve. In extension of the three-stage model, a parameter D_{MCI} was proposed that captures the curvature instability induced by a detergent. The MCI parameter revealed that LPC12 is a strong ($D_{\mathrm{MCI}} > 1$) and LPC14 and LPC16 are weak ($D_{\mathrm{MCI}} < 1$) detergents of POPC membranes. From phenomenological modeling, we found that the parameter D_{MCI} can be expressed by the bending rigidity, $cmc \cdot K$, and the local mean curvature coupling-parameter λ, which is related to the packing properties of LPCs in the bilayer. The criterion of a strong detergent by the model of Heerklotz and Seelig (6) ($K \cdot cmc < 1$) is not always sufficient, and the introduction of the D_{MCI} parameter may provide an alternative determination of detergent strength.

The MEMPHYS-Centre for Biomembrane Physics is supported by the Danish National Research Foundation. J.R.H. was supported by the Danish Medical Research Council (grant No. 271-08-0146) and the Danish Council for Strategic Research, Nanoscience and Technology, Biotechnology and IT (NABIIT) (grant No. 2106-07-0033). L.N.F. was supported by the Danish Council for Strategic Research, NABIIT (grant No. 2106-08-0081).

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