Scientific contribution/Original research

Nonspecific Influence of Surfactin on Lipid Membranes is Temperature Dependent

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Abstract:
Surfactin is a lipopeptide produced by bacillus subtilis, and is a membrane fusion inhibitor that has a strong anti-viral effect. Surfactin has shown to solubilize cell and model membranes with a detergent-like mechanism that is highly unspecific. Unlike typical detergents its peptide headgroup is partially hydrophobic, resulting in a deeper insertion into the hydrophobic tail region of model membranes, which may be the reason for its high activity and specific, chain-tilting effects on membrane order. In this work we exposed dipalmytoyl-oleoylphosphatidylcholine (DOPC) giant unilamellar vesicles (GUVs) to different concentrations of Surfactin and found that at room temperatures, the membranes are stabilized with a network of pores resembling crater-like structures in optic and fluorescent microscopy images. At higher temperatures (60 °C), the pores disappeared and the vesicles were smooth like the control groups. We used a three-dimensional Monte Carlo scheme emulating the permeabilization process and found that simulations are a good predictor of experiments is we take into account the temperature-dependent spontaneous curvature of Surfactin. This is in line with previous experiments that show Surfactin disorders the acyl chains, rendering microdomain complexes on the membrane less negatively curved at an increased temperature.

Keywords: Giant phospholipid vesicles; Giant unilamellar vesicles; Surfactin; Lipid membrane; Monte Carlo simulation of vesicle shape; Membrane pore

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1. Introduction

Surfactin is a cyclic molecule with the characteristics of a wedge lipid: two acidic amino acid residues form a larger hydrophilic head, while the long chain fatty acid forms a smaller hydrophobic tail (Yuan et al., 2018). Surfactin has shown to solubilize cell and model membranes with a detergent-like mechanism that is highly unspecific. Unlike typical detergents its peptide headgroup is partially hydrophobic, resulting in a deeper insertion into the hydrophobic tail region of model membranes, which may be the reason for its high activity and and specific, chain-tilting effects on membrane order. Solid-state NMR studies of the structural effects exerted by surfactin show that only surfactin promotes a tilt of the acyl chains, behavior that is not observed in detergents (Heerklotz et al., 2004). However, the simple pizza-slice logic of a wedge-shaped Surfactin does not apply in reality. Since Surfactin promotes the tilting of the acyl chains of nearby lipids, a surfactin-lipid nanodomain can have net negative curvature. In this paper, this hypothesis is tested with optic and fluorescence microscopy and via a 3D Monte Carlo simulation.

2. Experimental observation

Giant DOPC unilamellar lipid vesicles were prepared as described by Moscho et al. (1996). Giant DOPC lipid vesicles were exposed to Surfactin under the microscope and monitored online to capture the early lipid vesicle dynamics. In the experiments, the concentration of GUV were between 106 and 107 vesicles/mL. All experiments were made under ambient conditions (room temperature, ambient air pressure) and higher temperatures (60 °C). The binary solutions were prepared with 9 μL of vesicle solution pipetted onto #1,5 microscope cover glass to form hemispheric drop, after positioning and focusing the solution on the microscope, approximately 1 μL of appropriate Surfactin solution final concentrations of Surfactin were approx. 0,2 mM. Image acquisition started right after the start of addition of surfactin. Dynamics of lipid solubilization with surfactin was visualized with laser microscope fluorescence microscope Zeiss Axio Observer Z1 equipped with confocal unit LSM 800 (**Figure 1**).

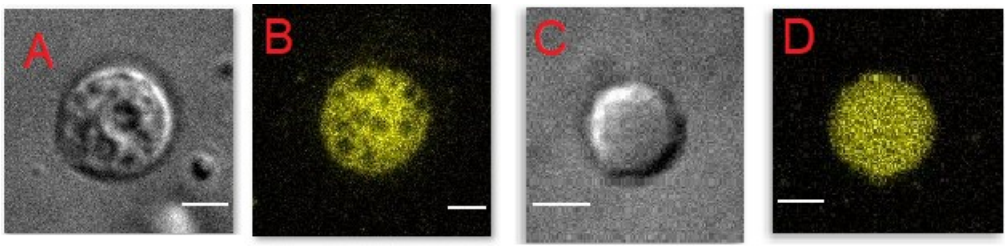

Figure 1. Surfactin and GUV microscopy results taken at room temperature (A, B) and at 60 °C (C, D). The vesicles are stable for many minutes and display no violent dynamics like in the case of detergent Triton X-100. The bar represents 5 μm. From (Drab et al., 2021).

3. Monte Carlo simulation results

The membrane is represented by a set of N vertices that are linked by tethers of variable length l to form a closed, dynamically triangulated, self-avoiding two-dimensional network (as described in (Gompper and Kroll, 1996; 2004; Fošnarič et al., 2019)). The microstates of the membrane are sampled according to the Metropolis algorithm. The probability of accepting the change of the microstate due to vertex move or bond flip is $\min[1, \exp(-\Delta E/kT)]$, where ΔE is the energy change, k is the Boltzmann constant and T is absolute temperature. The energy for a given microstate is specified by the standard Helfrich equation (Helfirch, 1973):

$$W_b = \frac{\kappa}{2}\int_A (c_1 + c_2 - c_0)^2 dA, \quad (1)$$

where the integral runs over the whole area of the membrane with bending stiffness κ, c_1 and c_2 are principal curvatures and c_0 the spontaneous curvature of the surfactin inclusions. The surfactin inclusions on the membrane are therefore modeled as patches of the membrane with given spontaneous curvature c_0. The patches occupied by the surfactins we set $c_0 < 0$ and elsewhere we assume a symmetric membrane $c_0 = 0$.

Additionally, to account for associative nature of membrane inclusions, a step potential between neighboring curved inclusions is taken into account by an additional energy term:

$$W_d = -w \sum_{i<j} \mathcal{H}(r_0 - r_{ij}), \tag{2}$$

where w is a direct interaction constant, the sum runs over all surfactin-surfactin pairs, r_{ij} are their mutual in-plane distances, $\mathcal{H}(r)$ is the Heaviside step function and r_0 is the range of the direct interaction. We consider here attractive interactions $w > 0$ that induce phase-separation of the lipid bilayer.

In this work we set N_d of the total $N = 1447$ vertices to represent surfactin domains (curved inclusions), which have spontaneous curvature c_0 that can be described well by the discrete mesh. All other vertices represent symmetric membrane and have zero spontaneous curvature. The positive sign of c_0 for curved inclusions indicates a tendency to curve the membrane outwards. The density of curved inclusions on the membrane is given by a fraction:

$$\rho = \frac{N_d}{N}. \tag{3}$$

We presume that surfactin has a temperature-dependent spontaneous curvature. We approximate this function by setting

$$c_0 = \frac{1}{2}\left(\frac{T}{T_0}\right) - 1. \tag{4}$$

In each such step, the total energy $W = W_b + W_d$ is numerically minimized. We can also define the mean cluster size of nanodomains with the equation:

$$\langle N_{vc} \rangle = \langle \frac{\sum N_{vc}^i N_{cl}^i}{N_{cl}^i} \rangle. \tag{5}$$

Here, the angle brackets denote the canonical ensemble average. At any given time during the simulation, N_{vc} is the mean cluster size and the sums run over all clusters of vertices representing proteins. In the sums N_{vc}^i is the number of vertices in cluster i and N_{cl}^i is the number of clusters of size N_{vc}^i. The cluster increase in size with increasing density of surfactin inclusions.

Simulation results shown in **Figure 2** reveal that the phase space of solutions in the (ρ,(T/T_0)) plane results in smooth vesicles, while at lower temperatures, a transition towards buds is seen (marked by the S-E transition line). At lower temperatures, more of these buds are seen (E(4) means that 4 buds are seen in the simulation). Some of these simulated shapes are shown in **Figure 3**.

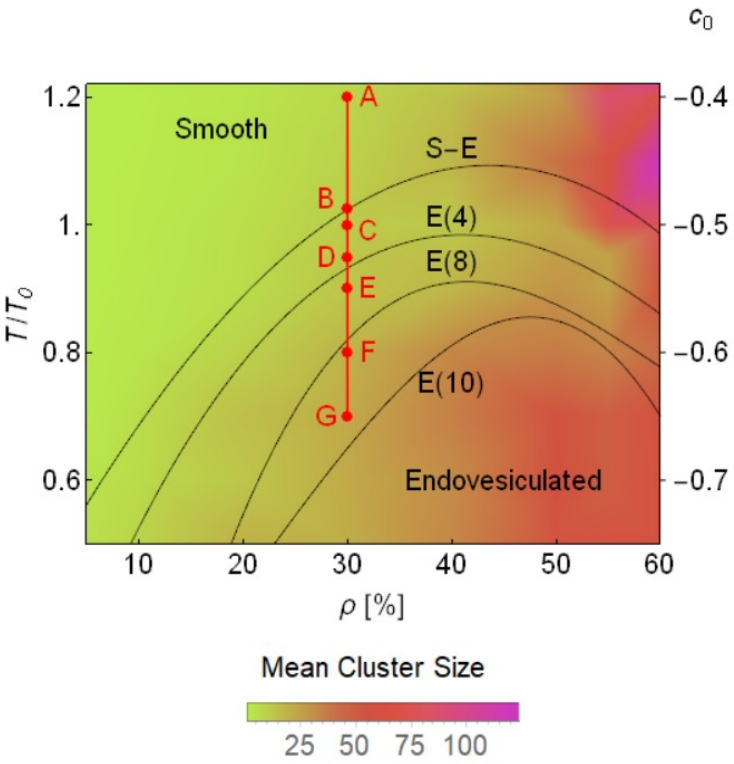

Figure 2. A phase diagram of the simulations in the (ρ,(T/T_0)) plane. Note that increasing temperature corresponds to less negative net spontaneous curvature of the nanodomains. The lines denote regions where inward curving buds are observed that correspond to crater like shapes in microscopic images. The cross-sections of the final micorstates of the vesicles shown in the slice of the phase diagram denoted by the red line are seen in **Figure 3**. The color scheme represents the mean cluster size given by Eq.(4).

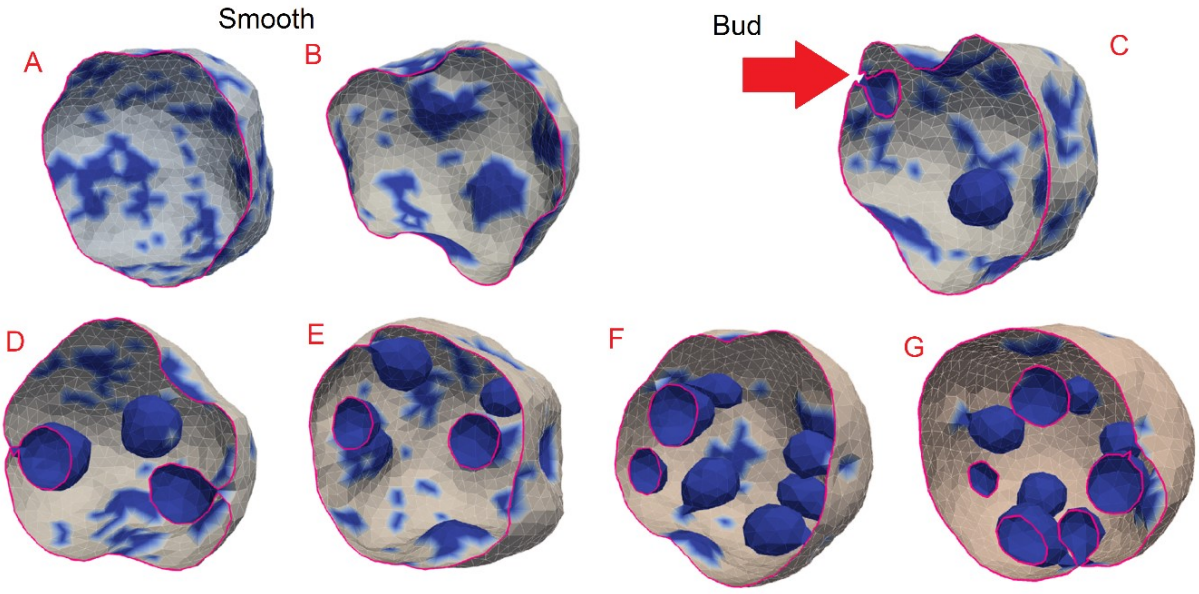

Figure 3. Cross sections of final microstates of vesicles for points shown in **Figure 2**. The patches of flat membrane with no spontaneous curvature are shown gray, while the blue areas correspond to negative spontaneous curvature c_0 where curved inclusions are present.

4. Discussion

Solubilization mechanisms of Surfactin are not completely understood and their use remains empirical even for systems of liposomes. The structural changes of liposomes induced by detergent solutions are known experimentally for some time and reveal that liposomes take various types of solubilization pathways depending on the types of lipids and detergents (Arnulphi et al., 2007; Tomita et al., 2011). In the present work, a possible mechanism of membrane structural changes seen in experiments with DOPC liposomes and Surfactin is presented within a simple Monte Carlo model of curved inclusions that can move over the membrane laterally and induce local curvature changes due to their molecular shape. This leads to a temperature dependent shapes seen in experiments before total solubilization and micellization of liposomes takes place. This is due to the unspecific surfactin mechanism that tilts the acyl chains at higher temperatures, rendering the net spontaneous curvature of the nanodomains less negative.

5. Conclusions

In this work the interaction between DOPC GUVs and Surfactin was studied with an emphasis on the processes prior to solubilization. An interesting temperature dependence of pore foramtions was observed at room temperature and 60 °C. A possible mechanism for such a process was proposed that is based on the geometrical and associative properties of the surfactin molecules that are adsorbed and laterally diffuse across the lipid vesicle. A 3D Monte Carlo numerical simulations were used to study the phase space of stable shapes and their spontaneous curvature dependence on temperature explored. It was found that at lower temperatures, the nanodomains form to construct a highly ordered membrane with buds that curve inward, while at higher temperatures, the more fluid membrane results in a less net negative curvature, rendering the shapes smoother. The results are in line with the existing literature and shed a new light on the mechanical and dynamical aspects of the early stages of the solubilization process.In his view the problem is ill-posed. He was asked to sing the love as if it were a single thing, then there are several types of love. We must look what kind of love is worthy of praise.

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Conflicts of Interest: The authors declare no conflict of interest.

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