Membrane Solubilization by Detergent: Resistance Conferred by Thickness

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The commonly held model for membrane dissolution by detergents/surfactants requires lipid transport from the inner to the outer bilayer leaflet (flip-flop). Although applicable to many systems, it fails in cases where cross-bilayer transport of membrane components is suppressed. In this paper we investigate the mechanism for surfactant-induced solubilization of polymeric bilayers. To that end, we examine the dissolution of a series of increasingly thick, polymer-based vesicles (polyerosomes) by a nonionic surfactant, Triton X-100, using dynamic light scattering. We find that increasing the bilayer thickness imparts better resistance to dissolution, so that the concentration required for solubilization, after a fixed amount of time, increases nearly linearly with membrane thickness. Combining our experimental data with a theoretical model, we show that the dominant mechanism for the surfactant-induced dissolution of polymeric vesicles, where polymer flip-flop across the membrane is suppressed, is the surfactant transport through the bilayer. This mechanism is different both qualitatively and quantitatively from the mechanisms by which surfactants dissolve pure lipid vesicles.

Introduction

The study of membrane-surfactant (detergent) interactions is driven by the need to solubilize biomembranes for the isolation, purification, reconstitution and crystallization of membrane proteins,1–5 or for the incorporation of pharmacological agents into synthetic drug-carrying vesicles.6,7 To understand the mechanisms governing membrane dissolution, the solubilization behavior of model vesicles has been extensively investigated.1–12 Generally, it has been shown that the solubilization process proceeds through a three-stage procedure with the characteristics of a phase transition.5,6,17–22 In the first stage, detergent is incorporated into the membrane, but the vesicle (or cell) remains intact. Once the surfactant concentration in the bilayer exceeds a critical value, mixed micelles containing membrane components and detergent detach. This process is associated with trans-bilayer transport (flip-flop) of lipids from the inner to the outer membrane leaflet. The second stage therefore consists of detergent-saturated membranes coexisting with mixed micelles. In the last stage all membrane components are fully solubilized into mixed micelles. The type of the transition depends on the detergent type:2 for nonionic surfactants, bilayer composition reaches saturation and then remains constant during the dissolution into micelles, reminiscent of a second-order transition. In the case of ionic surfactants, the bilayer disintegrates rapidly in a first-order like manner when the surfactant concentration reaches a critical value.


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Figure 1. Solubilization of giant (EO12−BD126) vesicles using Triton X-100. The polymer concentration is 0.1 mg/mL and Triton X-100 concentration is 12.5 mM. The numbers denote the time passed, in seconds, since the addition of the surfactant. Scale bar is 6 μm. We see that before the addition of surfactant (t = 0), the polymersome is spherical. Upon the addition of surfactant, ‘fingering’ develops, indicating the creation of excess area. However, the polymersome remains intact until t ≈ 10 s, where rupture takes place (indicated by loss of phase contrast that corresponds to sucrose leakage from the interior). It is interesting to note that even after rupture segments of the polymeric bilayer persist, taking longer to disintegrate.

Table 1. Comparison of Membrane Thickness, L, and Molecular Weight of Diblock Copolymers Used in the Solubilization Study: EO, Ethylene Oxide; EE, Ethyl Ethylene; BD, Butadiene; Li, Hydrophobic Membrane Thickness; Mw, Number-Averaged Molecular Weight.

<table>
<thead>
<tr>
<th>Vesicle Name</th>
<th>Formula</th>
<th>( L (\text{nm}) )</th>
<th>( M_w (\text{g/mol}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>OE7</td>
<td>EO80−EE37</td>
<td>8.0</td>
<td>3900</td>
</tr>
<tr>
<td>OB2</td>
<td>EO26−BD86</td>
<td>9.6</td>
<td>3600</td>
</tr>
<tr>
<td>OB18</td>
<td>EO12−BD126</td>
<td>14.8</td>
<td>10400</td>
</tr>
</tbody>
</table>

The three-stage solubilization model has been successfully applied to nonionic surfactant systems such as Triton X-100, octyl glucoside, and C12EO2. However, there is strong evidence\(^3\)\(^7\)\(^10\)\(^20\)\(^23\) that other pathways exist, depending on the state and nature of the detergent and the membrane. Moreover, it is obviously applicable to systems where membrane component transport across the bilayer is suppressed.

The goal of this paper is to investigate pathways for surfactant-induced membrane solubilization. Specifically, we focus here on the role of the amphiphile transbilayer mobility and its effect on dissolution process. To that end, we utilize block copolymer vesicles where the bilayer thickness, and the transbilayer mobility, may be controlled through the polymer molecular weight.

Polymeric vesicles, or ‘polymersomes’, have been recently used to explore fundamental and technological issues relating to membrane self-assembly and behavior.\(^2\)^\(^6\)^\(^24\) These polymeric vesicles self-direct their assembly from hydrophilic-hydrophobic copolymers, commonly composed of water-favoring poly(ethylene glycol) attached to hydrocarbon chains.\(^26\)^\(^32\)^\(^33\)

The polymer’s high molecular weight (when compared to lipids) imparts increased stability and strength to the membrane, and slows the kinetics of various processes, thereby allowing systematic studies.\(^25\)^\(^26\)^\(^28\)

We explore the solubilization of polymeric bilayers by a nonionic surfactant, Triton X-100, using primarily dynamic light-scattering, a technique that has been widely utilized for the study of lipid bilayer solubilization by detergents.\(^11\)^\(^12\) We find that the concentration of surfactant required for vesicle solubilization increases, nearly linearly with membrane thickness, Analysis of our results indicates a new solubilization mechanism dominated by surfactant transport through the bilayer, rather than by membrane component ‘flip-flop’.

Materials and Methods

Materials. Diblock copolymers were synthesized in the manner described in Hillmyer and Bates.\(^8\)^\(^9\) Table 1 shows the membrane thickness and molecular weight of the three different kinds of polymeric vesicles used in this study.

Chemicals. Phosphate-buffered saline (PBS), at a pH of 7.4 and concentration ~300 mM, was prepared by dissolving PBS tablets from Sigma (St. Louis, MO) in deionized water. Triton X-100 was purchased from Sigma (St. Louis, MO) and used without further purification.

Preparation of Polymer Vesicles. Preparation of polymeric vesicles was accomplished by film rehydration. In short, 100 μL of a 10 mg/mL polymer stock solution in chloroform was uniformly coated on the inside wall of a glass vial, followed by evaporation of the chloroform under vacuum for 3 h. Rehydration of the polymer film with 1 mL of an aqueous solvent (PBS at ~300 mM) led to spontaneous budding of vesicles off the glass wall into solution. Vesicle yield is further promoted by overnight incubation at 60 °C in an oven. In the case of giant vesicles (Figure 1), no further steps were taken. However, to obtain small vesicles for the DLS study, vesicle size was reduced to 100 nm by sonication, freeze–thaw cycles, and extrusion through a 0.1 μm polycarbonate filter.

Polymersome Solubilization. To study the solubilization of polymersomes, Triton X-100 detergent was homogeneously mixed with polymeric vesicles below and above its CMC of 0.25 mM (ranging from 0.1 mM to 250 mM). Samples were incubated for 10 min prior to measurement of intensity with dynamic light scattering technique. Solubilization studies were performed on three different types of polymeric vesicles that differ in membrane thickness as shown in Table 1.

Dynamic Light-Scattering (DLS) Measurements. The hydrodynamic radius of pure polymeric vesicles, pure Triton X-100 micelles and particles formed after mixing different concentrations of detergent with polymersomes were determined by means of a dynamic light-scattering (DLS) technique using a photon correlator spectrometer (DynaPro-LSR, ProteinSolutions, Lakewood, NJ). Quartz cuvettes were filled with 50 μL samples, and all the experiments were thermostatically controlled at 23 °C. All the DLS measurements were made at a scattering angle of 90°. The analysis of the data was performed using DYNAMICS software provided by ProteinSolutions. The results are given as hydrodynamic radii (HR), and the percentages correspond to intensity values.
Results

In Figure 1 we show the evolution of a (giant) polymersome as it dissolves by incorporating Triton X-100. After a short incubation time, a spherical polymersome displays “fingering” or puckering indicative of excess area and, possibly, area mismatch between the inner and outer bilayer leaflets. The puckered condition persists for a period of time until rupture takes place (as seen from loss of phase contrast). However, even after rupture segments of the polymer membrane can still be seen.

DLS on smaller (∼100 nm) vesicles was used to obtain quantitative data on the dissolution process. Specifically, we obtained the hydrodynamic radius size distribution as a function of the surfactant concentration. In Figure 2 we show the distribution curves taken 10 min after the addition of surfactant, at a scattering angle of 90°. The curve for pure polymeric vesicles (C_s = 0) shows a distribution with a hydrodynamic radius (R_h) of order 70–110 nm, a value closely corresponding to our goal of 50 nm. In systems containing a low surfactant concentration (C_s = 5 mM), we see a bimodal distribution of large polymersomes and surfactant micelles. The latter are characterized by a peak at 4–10 nm, in agreement with previous observations of Triton X-100 micelles. As the concentration of the surfactant increases a new peak in the size distribution curve is detected: we infer that this intermediate population corresponds to polymer-surfactant mixed micelles, since the R_h of this new species, 30–40 nm, is consistent with that of a mixed polymer-surfactant micelle. Increasing surfactant concentrations above this critical value leads to a decrease in the polymersome intensity and a progressive rise in the intermediate population. Finally, as we exceed a second critical concentration, complete solubilization of vesicles is achieved (as evidenced by the disappearance of the vesicle peak) where we see pure surfactant micelles and a wide distribution of intermediate structures.

As shown in Figure 2, the solubilization of vesicles in polymersome/surfactant mixtures can be assessed through a decrease in the intensity of the polymersome peak. As the surfactant concentration is increased, more polymersomes are expected to solubilize and the scattering intensity of the polymer vesicles should therefore decrease. In Figure 3 we plot the intensity of the polymersome peak, 10 min after the addition of surfactant, as a function of the Triton concentration, for all three systems examined (as described in Table 1). We see that the process may be divided into roughly three regimes: at low surfactant concentrations (on the order 2 mM or less) the polymersome peak remains unaffected. Comparison to Figure 2 shows that in this regime the polymersomes coexist with Triton X-100 micelles. As the Triton concentration increases above a given value, a second regime is entered, where the peak intensity decreases with increasing surfactant concentration. This regime (see Figure 2) corresponds to the appearance and growth of mixed micelles. Finally, when the surfactant concentration increases further we enter the third regime where the polymersomes have disintegrated. In this regime, Triton micelles and mixed micelles coexist, possibly with membrane fragments (see Figure 1). Thus, the process of polymersome solubilization may be characterized through two critical surfactant concentrations: the concentration at which mixed micelles appear, and/or the concentration at which complete dissolution takes place. Unfortunately, it is somewhat difficult to accurately determine these specific values. We therefore chose as a criteria the surfactant concentration required for a 50% attenuation in the intensity of the vesicle peak. This attenuation can be taken as an indication of 50% solubilization of the polymersomes. In Figure 4 we plot the value required for 50% polymersome solubilization, C*_s, as a function of

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membrane thickness, \( L \). We see that the surfactant concentration required to solubilize a fixed fraction of the vesicles increases, nearly linearly with membrane thickness.

**Discussion and Conclusions**

We investigate here the dissolution of (uncharged) polymeric vesicles by nonionic surfactants. We find that at short periods of time and/or surfactant concentrations (Figures 1–3) the polymersomes coexist with surfactant micelles, although they display an increase in surface area (“puckering”). Above a critical surfactant concentration, the polymersomes start to disintegrate, and a population of mixed polymer-surfactant micelles appears. At higher surfactant concentrations, all polymersomes have dissolved and we see only mixed micelles coexisting with surfactant micelles and, possibly, membrane fragments (Figures 1–3).

As mentioned in the Introduction, lipid-based vesicles dissolve through adsorption and incorporation of surfactant into the external leaf of the bilayer, followed by ejection of mixed lipid–surfactant micelles. The “loss” of lipids from the outer membrane leaflet leads to an area asymmetry between the inner and the outer leaflets, which is relieved by the migration of lipids from the inner to the outer monolayer, i.e., “flip-flop.” It should be noted that although lipid flip-flop is often actively suppressed in cell membranes, it can be quite significant in equilibrium systems.\(^{35,36}\)

In the case of polymeric bilayers, the polymer chains in the bilayer are stretched due to crowding at the hydrophobic/hydrophilic interface.\(^{37}\) This crowding can be relieved by the incorporation of surfactant molecules, a driving force that is stronger than the simple mixing entropy dominating the interactions between nonionic surfactants and uncharged lipid bilayers. The stages of polymersome dissolution can be summarized as follows (see Figure 5): as surfactant molecules are incorporated into the outer leaflet of the polymeric bilayer, an area imbalance between the outer and inner leaflets evolves. This imbalance may be relieved by either (1) flip-flop of the polymer chains from the inner to the outer leaflet, (2) detachment of polymer-surfactant micelles from the outer leaflet, and/or (3) diffusion of the surfactant molecules, through the hydrophobic core, from the outer to inner leaflet, followed by micelle detachment from both leaflets.

The first option is clearly suppressed; flip-flop of polymeric chains between the leaflets requires diffusion of copolymer chains through the hydrophobic membrane core. As shown by Hamersky et al.,\(^{38,39}\) the diffusion of a diblock copolymer through lamellar domains (i.e., in the perpendicular direction) is largely suppressed.

Let us examine the second possibility: ejection of mixed polymer-surfactant micelles requires that the concentration of surfactant in the outer membrane exceed a critical value, which is set by the mixed polymer-surfactant micelle composition. In the case of pure block copolymer micelles, the aggregation number is set by a balance between the hydrophobic block stretching energy and the interfacial tension between the hydro-
phobic and hydrophilic regions, the former favors a low aggregation number, while the latter drives for larger aggregates. In the case of the polymer-surfactant micelles, the surfactant reduces the interfacial tension in a manner similar to that of amphiphiles stabilizing oil droplets in aqueous solutions. Thus, when the surfactant is in excess, we may assume that the mixed polymer-surfactant micelles contain only a few polymer chains so as to minimize hydrophilic chain stretching. The micelles core is composed of collapsed hydrophobic blocks and stabilized by a monolayer of surfactant. This assumption is largely consistent with the hydrodynamic radius (Rh) values we obtain for the mixed structures.

Consider, then, a solution of a diblock copolymer whose overall molecular weight is $N$ and its concentration is $C_p$ (weight/vol). We define the diblock composition through the molecular weight fraction of the hydrophobic block $\gamma_c$. The mixed polymer-surfactant micelle core radius is set by the volume of the collapsed polymer hydrophobic blocks: $R \sim a f^{1/3} (\gamma_c N)^{1/3}$, where $a$ is a segment size and $f$ the aggregation number. The number of surfactant molecules required to stabilize a droplet of radius $R$ is given by $R^2 / \Sigma$, where $\Sigma$ is the interfacial area per surfactant molecule and is assumed, for simplicity, to be independent of curvature. Thus, to solubilize a given number of polymer chains we need a surfactant concentration of

$$C^*_s = \frac{a^2 C_p \gamma_c^{2/3}}{\Sigma N^{1/3} f^{1/3}}$$

(1)
Thus, for copolymers of similar asymmetry ($y_i$ fixed), aggregation numbers ($f_i$), and polymer concentration $C_p$, the surfactant concentration required for solubilization scales as $N^{-1/3}$. This trend does not agree, even qualitatively, with our data where the concentration required for polymersome dissolution increases, with chain length (see Figure 4, recalling that the core thickness $L$ scales with $N$).

Let us examine the third mechanism, whereby polymersome dissolution is dominated by surfactant transport from the outer to inner leaflet. We therefore need to calculate the surfactant flux and concentration in the interior leaflet of the membrane as a function of the bilayer thickness and the surfactant concentration in solution. Assume a simple diffusion model of the form

$$\frac{\partial C_s}{\partial t} = D \frac{\partial^2 C_s}{\partial x^2}$$

(2)

where $t = $ time, $D = $ diffusivity of surfactant across the membrane, and $x = $ distance from the outer leaflet of the membrane. Solving the above equation (assuming surfactant accumulation in the inner leaflet) we obtain the following expression for the surfactant concentration in the inner leaflet:

$$C_s(L) = HC_0 [1 - e^{-\frac{2DL}{4L^2}}]$$

(3)

where $L$ is the membrane thickness and $C_0$ is the surfactant concentration in solution. $H$ is the partition coefficient for the surfactant between water and the bilayer; in our discussions we will take it to be of order unity.$^{44}$

In this scenario, polymersome dissolution will take place when the surfactant concentration in the inner leaflet reaches the critical concentration required to form mixed micelles calculated in eq 1. Using the relationship between the polymer molecular weight and the core thickness$^{40}$ $L \sim a(y_i)N_i^{2/3}$, we can determine the surfactant concentration in solution necessary to obtain $C_s^*$ in the inner leaflet as

$$C_s^* = \frac{\alpha^2 C_p y_i}{\Sigma L^{1/2}} [1 - e^{-\frac{2DL}{4L^2}}]$$

(4)

so that, in this model, solubilization of all polymersomes (at a fixed time $t$) will be obtained when the surfactant concentration $C_0$ is given by $C_s^*$.

Examining eq 4 we see that $C_s^*$ is a function of only two unknown lumped parameters: $(\alpha^2/2\sqrt{\gamma_i/\Sigma L^{1/2}})$ and $(\alpha^2 D)$. Assuming that the fraction of dissolved polymersomes $p$ is set by a simple proportionality, $p \sim C_0/C_0^*$, we plot in Figure 6 the data obtained for the fraction of dissolved polymersomes ($p$) as a function of the core thickness $L$ and the surfactant concentration in solution $C_0$. We see that, for a given surfactant concentration, the fraction of polymersomes dissolved (after a set $t = 10$ min) decreases mildly with $L$. For a given layer thickness, the fraction of dissolved polymersomes increases monotonically with $C_0$. Using eq 4 and a two-parameter fit for $p$, we see a very good agreement between the model predictions and the experimental data. Note that this fit is quite stringent; it implies that the surfactant diffusion rate through the two types of copolymers is similar, and that the mixed micelle aggregation number is insensitive to the polymer molecular weight. Thus, fitting 12 data points (Figure 6) with only two parameters is a significant validation of the model.

The results of our study clearly show the dominant mechanism for the surfactant-induced dissolution of polymeric vesicles is the surfactant transport through the polymeric bilayer. This mechanism is different, both qualitatively and quantitatively, from the mechanisms by which surfactants dissolve lipid-based vesicles,$^{5,6,17-22}$ and arises from the suppression of polymer flip-flop from the inner to the outer membrane leaflet.$^{38,39}$ We conclude that polymeric bilayers cannot be treated as ‘thicker lipid bilayers’, but display unique features arising from their polymeric nature that are more typical of large glycolipids or membrane proteins.

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