Surfactant-Induced Lysis of Lipid-Modified Microgels

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Lipid layers supported on hydrophilic polymer cushions have attracted considerable attention, not only because they provide a cell-like environment for transmembrane protein incorporation, but also because the lipid membrane creates a thin barrier that is able to maintain a chemical gradient. These properties have inspired the preparation of novel types of hybrids that combine lipids and hydrogels. For sensory applications, a simple method for the integration of these objects within a microfluidic device and a means to chemically trigger ion barrier disintegration would be highly advantageous. Previously, we described the in situ formation of a hydrogel object within a microchannel that was constrained by the glass channel at their top and bottom interfaces. The polymer gel consisted of 2-hydroxyethyl methacrylate, acrylic acid (20 vol %), and ethylene glycol dimethacrylate (1 vol %) photoinitiated with 2,2-dimethoxy-2-phenylacetophenone (3 wt %). To monitor the pH inside of the polymer gel, a pH-sensitive indicator (phenolphthalein or fluorescein) was entrapped within the hydrogel matrix during polymerization. The dried gel was bathed in benzene and esterified with palmitoyl chloride, resulting in the covalent attachment of a fatty acid layer to the polymer gel surface. This modification procedure creates an ion barrier that enables a pH-sensitive gel to remain contracted while bathed in a pH solution that otherwise expands an unmodified gel. By analogy to the lyzing behavior of surfactants on cells and vesicles, we investigated the ability of the ionic surfactant sodium dodecyl sulfate (SDS) and the nonionic surfactant Triton X-100 to disrupt the lipophilic ion barrier.

Prior to the addition of detergent, each modified gel was exposed to an elevated buffer (pH 12) for a few hours to demonstrate that the fatty acid layer was impermeable to ions, as evidenced by the lack of gel swelling. A solution of the surfactant dissolved in a pH 12 buffer was then flowed into the channel, and the diameter of the gel was measured as a function of time. At surfactant concentrations above the critical micelle concentration (cmc) (1 mM for SDS, 0.24 mM for TX-100), localized regions of expanded hydrogel were visible within minutes at the surface of the object. These areas grew larger until the entire gel expanded and the phenolphthalein indicator changed from colorless to pink (Figure 1). At lower surfactant concentrations, the behavior was similar but surface disruptions appeared more slowly.

The pH change within the gel was imaged with confocal microscopy to determine if surface disruptions initially formed at the gel–glass interface or in the gel interior. This was accomplished by monitoring the increase in the emission intensity of the pH-sensitive fluorescein dye entrapped in the gel. For an unmodified gel, the emission increase, and therefore gel expansion caused by the inward diffusion of buffer, began uniformly on the surface and symmetrically progressed to a point at its center (Figure 2a–d). In contrast, when a fatty-acid modified gel was exposed to a surfactant solution, buffer permeation began in a localized site (e), which propagated unsymmetrically (f, g) until the buffer diffused throughout the gel (h). The scale bar is 200 μm. A digital movie of these experiments is available as Supporting Information.

Figure 1. A pH sensitive hydrogel was modified by covalently linking palmitoyl chloride to the surface. When the gel was bathed in a pH 12 buffer solution, it remained stable for hours (●) while an unmodified gel rapidly expanded in the same solution (●). The addition of a 0.1 M solution of SDS in a pH 12 buffer to the modified gel at the indicated time (○) triggered localized areas of expansion on the exterior of the gel (a), which propagated to adjacent regions (b) until the entire surface of the gel expanded (c). Full gel expansion was complete when the pH indicator phenolphthalein changed from colorless to pink at the interior of the gel. f0 is the fractional change in diameter, ΔD, where ΔD is the total diameter change for the fully expanded gel. The scale bar is 250 μm.

Figure 2. Buffer diffusion into a fluorescein-loaded gel was monitored with a confocal microscope. The presence of elevated pH solution within the gel was signaled by an increase in fluorescence emission. With an unmodified gel, expansion began at the exterior of the gel (a) and moved inward (b, c) until it reached the center (d). In contrast, when a fatty-acid modified gel was exposed to a surfactant solution, buffer permeation began in a localized site (e), which propagated unsymmetrically (f, g) until the buffer diffused throughout the gel (h). The scale bar is 200 μm. A digital movie of these experiments is available as Supporting Information.

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(5) See Supporting Information for experimental details.
surfactant addition, ion permeation began at localized regions, as signaled by a localized increase in fluorescence intensity and \( \mu \) gel expansion (Figure 2e). Undulation of the \( \mu \) gel surface originated at an intermediate channel depth, indicating that delamination of the \( \mu \) gel from the glass channel was not the mode of buffer entry. Initial expansion increased the \( \mu \) gel’s surface area, which apparently lowers the density of the covalently attached fatty acids around the site of the perturbation and further increases ion permeability in this area, as schematically shown in Figure 3. As a result, ion permeation propagated around the object’s circumference (Figure 2f) until the entire fatty acid layer was permeable to the buffer (Figure 2g), and ultimately complete \( \mu \) gel expansion accompanied by an increase in fluorescence intensity occurred (Figure 2h). As a consequence of the unsymmetric ion permeation around the fatty acid layer, the final region of the \( \mu \) gel exposed to buffer was off-center, biased toward the side furthest from the initial undulation.

To investigate how the rate of \( \mu \) gel expansion depends on surfactant concentration, 10 trials at each concentration of SDS and TX-100 were performed. The time interval for each \( \mu \) gel to reach a fractional change in diameter \( f_D \) of 1/e after addition of the surfactant solution was estimated from a graph of time vs \( f_D \), and the mean time and 95% confidence values were determined for each set of 10 runs with standard statistical analysis methods (Figure 4). Although we did not find a correlation between the number of localized perturbations initially observed on the \( \mu \) gel and the surfactant concentration, the time interval for \( \mu \) gel expansion depended on both the concentration and nature of the surfactant. Modified \( \mu \) gels exposed to TX-100 expanded slightly faster than those exposed to a SDS solution with the same concentration, which is consistent with the previously reported effects of these detergents on lipid membranes. However, for both surfactants, the average rate of expansion vs the surfactant concentration decreased rapidly below the cmc.

The results shown in Figure 4 are similar to those reported for synthetic lipid membranes and suggest that the mechanism of surfactant-induced \( \mu \) gel expansion follows a related process,\(^8\)\(^9\) wherein nonmicellar surfactant molecules adsorb onto the lipid membrane and induce ion permeability prior to membrane solubilization.\(^10\) Similarly, upon exposure of the fatty acid modified \( \mu \) gel to a surfactant solution, nonmicellar surfactant molecules adsorb onto the lipophilic \( \mu \) gel surface. Though the specific details are as of yet unknown, ion permeability increases

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**Figure 3.** Postulated process for the propagation of regional ion permeability induced by surfactants. A localized perturbation (a) causes an increase in surface area, lowering the local fatty acid chain density (b). \( \mu \) gel expansion propagates to neighboring regions by ion diffusion through the hydrogel (c, d). Eventually, the buffer infiltrates the entire \( \mu \) gel circumference.

**Figure 4.** Plot of the average time for \( \mu \) gel expansion to reach \( f_D = 1/e \) for SDS (■) or TX-100 (□) vs the surfactant concentration, including the 95% confidence values for each point (solid line). Averages were determined from 10 runs.

in a localized region, which sets in motion the progression of steps shown in Figure 3. This nucleation event may, for example, involve the formation of a critically sized cluster of surfactant molecules. For concentrations above the cmc, the concentration of nonmicellar surfactant molecules in solution is buffered by micelle formation so it remains equal to the cmc.\(^9\)\(^10\) Thus, the rate of surfactant absorption onto the fatty acid layer, and therefore the rate of \( \mu \) gel expansion, does not vary appreciably above the cmc. However, below the cmc, the concentration of nonmicellar surfactant is nearly identical to the stoichiometric surfactant concentration. Therefore the rate of adsorption onto the fatty acid layer decreases as the concentration drops, and subsequently, the rate of \( \mu \) gel expansion slows significantly.

In conclusion, we have shown that the lipophilic \( \mu \) gel barrier can be chemically disrupted through the addition of surfactant to the bathing solution in a manner reminiscent of surfactant-induced cell lysis. Barrier disruption liberates the hydrogel’s chemical potential, and by a process that resembles nucleation and growth, an unsymmetric \( \mu \) gel expansion takes place. The fatty acid layer around the \( \mu \) gel perimeter is thus of sufficient thickness to establish a pH gradient, yet is thin enough to allow surfactant-induced breakdown. The ability of these objects to maintain or abolish a chemical gradient may provide a means to amplify weak chemical signals, since a small, localized perturbation can trigger the hydrogel’s “none-to-all” expansion.

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**Supporting Information Available:** Experimental details for channel fabrication, \( \mu \) gel synthesis, surface modifications, and expansion experiments (PDF) and digital movies of LSCM experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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