Surfactant-Induced Lysis of Lipid-Modified Microgels

Mary L. Kraft and Jeffrey S. Moore*

The Beckman Institute for Advanced Science and Technology
The University of Illinois at Urbana-Champaign
Urbana, Illinois 61801

Received August 30, 2001

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.[1] These properties have inspired the preparation of novel types of hybrids that combine lipids and hydrogels.[2] 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 creation of a hydrogel object within a microchannel that was covalently modified with a thin ion-impermeable fatty acid layer.[3] Herein, we report that the barrier permeability can be chemically induced with a surfactant solution, triggering the complete expansion of the hydrogel by an unusual process.

pH-sensitive hydrogel cylinders (μgels), 180 μm tall by 400 μm in diameter, were photopolymerized within microchannels following our reported method.[4] They were constrained by the glass channel at their top and bottom interfaces. The μgel copolymers consisted of 2-hydroxyethyl methacrylate, acrylic acid (4:1 vol ratio), and ethyleneglycol dimethacrylate (1 vol %) photoinitiated with 2,2-dimethoxy-2-phenylacetophenone (3 wt %). To monitor the pH inside of the μgel, a pH-sensitive indicator (phenolphthalein or fluorescein) was entrapped within the hydrogel matrix during polymerization. The dried μgel was bathed in benzene and esterified with palmitoyl chloride, resulting in the covalent attachment of a fatty acid layer to the μgel surface.[5] This modification procedure creates an ion barrier that enables a pH-sensitive μgel to remain contracted while bathed in a pH solution that otherwise expands an unmodified μgel. By analogy to the lysing 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 (TX-100) to disrupt the lipophilic ion barrier.

Prior to the addition of detergent, each modified μgel 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 μgel swelling. A solution of the surfactant dissolved in a pH 12 buffer was then flowed into the channel, and the diameter of the μgel was measured as a function of time. At surfactant concentrations above the critical micelle concentration (cmc) (1 mM for SDS,[6] 0.24 mM for TX-100[7]), localized regions of expanded hydrogel were visible within minutes at the surface of the object. These areas grew larger until the entire μgel 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.

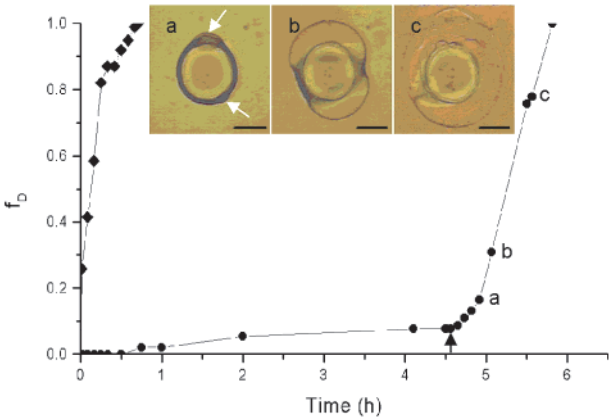

Figure 1. A pH sensitive hydrogel was modified by covalently linking palmitoyl chloride to the surface. When the μgel was bathed in a pH 12 buffer solution, it remained stable for hours (●) while an unmodified μgel rapidly expanded in the same solution (◆). The addition of a 0.1 M solution of SDS in a pH 12 buffer to the modified μgel at the indicated time (↑) triggered localized areas of expansion on the exterior of the μgel (a), which propagated to adjacent regions (b) until the entire surface of the μgel expanded (c). Full μgel expansion was complete when the pH indicator phenolphthalein changed from colorless to pink at the interior of the μgel. f_D is the fractional change in diameter, $\Delta d/d_0$, where d_0 is the total diameter change for the fully expanded gel. The scale bar is 250 μm.

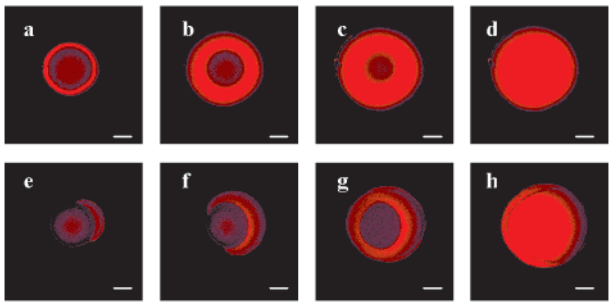

Figure 2. Buffer diffusion into a fluorescein-loaded μgel was monitored with a confocal microscope. The presence of elevated pH solution within the μgel was signaled by an increase in fluorescence emission. With an unmodified μgel, expansion began at the exterior of the μgel (a) and moved inward (b,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

The pH change within the μgel was imaged with confocal microscopy to determine if surface disruptions initially formed at the μgel−glass interface or in the μgel interior. This was accomplished by monitoring the increase in the emission intensity of the pH-sensitive fluorescein dye entrapped in the μgel. For an unmodified μgel, the emission increase, and therefore μgel 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, surfactant-induced expansion of the modified μgel proceeded unsymmetrically. Prior to addition of surfactant to the buffer solution, the fluorescein emission intensity was minimal and the μgel was contracted since the high-pH solution could not penetrate the μgel exterior. After

* To whom correspondence should be addressed.

(1) Sackmann, E. *Science* **1996**, *271*, 43−48.
(2) (a) Jin, T.; Pennefather, P.; Lee, P. I. *FEBS Lett.* **1996**, *397*, 70−74. (b) Ng, C. C.; Cheng, Y.; Pennefather, P. S. *Macromolecules* **2001**, *34*, 5759−5765. (c) Kiser, P. F.; Wilson, G.; Needham, D. *Nature* **1998**, *394*, 459−462.
(3) Beebe, D. J.; Moore, J. S.; Yu, Q.; Lui, R. H.; Kraft, M. L.; Jo, B.; Devadoss, C. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 13488−13493.
(4) Beebe, D. J.; Moore, J. S.; Bauer, J. M.; Yu, Q.; Lui, R. H.; Devadoss, C.; Jo, B. *Nature* **2000**, *404*, 588−590.
(5) See Supporting Information for experimental details.
(6) Wanless, E. J.; Ducker, W. A. *J. Phys. Chem.* **1996**, *100*, 3207−3214.
(7) Lichtenberg, D.; Robson, R. J.; Dennis, E. A. *Biochim. Biophys. Acta* **1983**, *737*, 285−304.

10.1021/ja016975g CCC: $20.00
Published on Web 12/19/2001

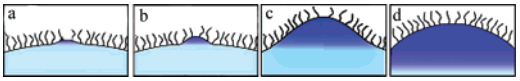

Figure 3. Postulated process for the propagation of regional ion permeability induced by surfactants. A localized perturbation (a) causes an increase in surface area, lowering the local fatty acid chain density (b). μgel expansion propagates to neighboring regions by ion diffusion through the hydrogel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

surfactant addition, ion permeation began at localized regions, as signaled by a localized increase in fluorescence intensity and μgel expansion (Figure 2e). Undulation of the μgel surface originated at an intermediate channel depth, indicating that delamination of the μgel from the glass channel was not the mode of buffer entry. Initial expansion increased the μgel'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 μgel 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 μgel exposed to buffer was off-center, biased toward the side furthest from the initial undulation.

To investigate how the rate of μgel expansion depends on surfactant concentration, 10 trials at each concentration of SDS and TX-100 were performed. The time interval for each μgel 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 μgel and the surfactant concentration, the time interval for μgel expansion depended on both the concentration and nature of the surfactant. Modified μgels 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.[8] 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 μgel 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 μgel to a surfactant solution, nonmicellar surfactant molecules adsorb onto the lipophilic μgel surface. Though the specific details are as of yet unknown, ion permeability increases

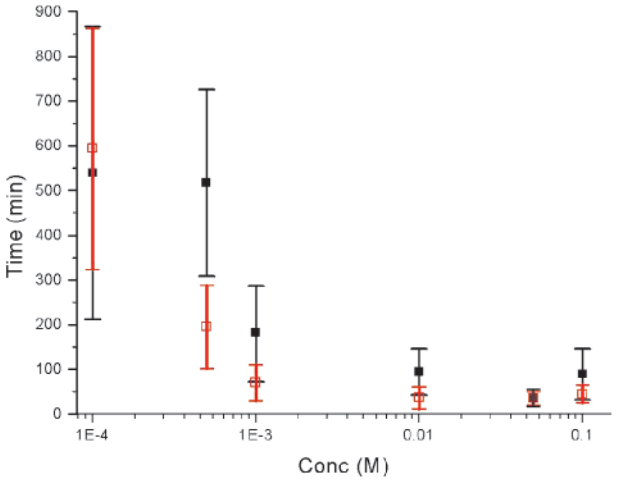

Figure 4. Plot of the average time for μgel expansion to reach $f_D = 1/e$ for SDS (■) or TX-100 (□) vs the surfactant concentration, including the 95% confidence values for each point (solid line). Averages were determined from 10 runs.

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 μgel 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 μgel expansion slows significantly.

In conclusion, we have shown that the lipophilic μgel 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 μgel expansion takes place. The fatty acid layer around the μgel 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.

Acknowledgment. This material is based upon work supported by the National Science Foundation (NSF DMR 01-06608) and DARPA-MTO.

Supporting Information Available: Experimental details for channel fabrication, μgel 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.

JA016975G

(8) Kragh-Hansen, U.; le Marie, M.; Moller, J. V. *Biophys. J.* **1998**, *75*, 2932−2946.
(9) Helenius, A.; Simons, K. *Biochim. Biophys. Acta* **1975**, *415*, 29−79.
(10) le Marie, M.; Champeil, P.; Moller, J. V. *Biochim. Biophys. Acta* **2000**, *1508*, 86−111.