Communication

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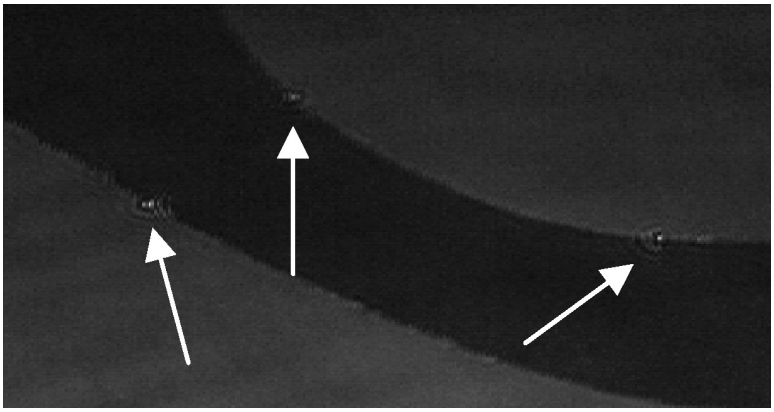

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Calcium Oxalate Monohydrate Precipitation at Membrane Lipid Rafts

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Calcium oxalate monohydrate (COM) is the major mineral component of most human urinary stones. The stone inorganic fraction is always mixed with an organic material composed of lipids, carbohydrates, and proteins, and studies have shown that lipid matrices, either synthetic models or isolated cellular media, can induce calcium oxalate precipitation.[1,2] Since urine is metastable with respect to COM, heterogeneous nucleation is the predominant precipitation process. Therefore, detailed understanding of how lipid assemblies can lead to calcium oxalate precipitation is highly relevant to the problem of urinary stone formation. Our group has recently shown that phospholipid phase boundaries play an important role in calcium oxalate precipitation when biphasic lipid monolayers are held at the air−water interface over a metastable calcium oxalate subphase.[3] Calcium oxalate monohydrate crystals were shown to preferentially form at phase boundaries between liquid-condensed (LC) and liquid-expanded (LE) phases.[3] Recent interest in the concept of lipid rafts draws attention to the fact that phase-separated heterogeneities are indeed present in cellular lipid membranes. We therefore investigated phase-separated assemblies containing lipid raft compositions and now show that COM precipitation from metastable solution occurs at the lipid raft phase boundaries. The observations highlight the possible role of ordered lipid assemblies in the urinary precipitation of calcium oxalate.

Monolayers, bilayers, and vesicles of composition derived from lipid rafts are often used as membrane models.[4−10] Common mixtures contain an unsaturated phosphatidylcholine, sphingomyelin, and cholesterol,[4−10] a makeup based on lipid composition analysis of Madin−Darby canine kidney (MDCK) cells and Triton-insoluble detergent-resistant membranes.[11] The MDCK cell composition is a pertinent membrane model for COM formation. Raft-forming monolayers were prepared from a 2:1:1 mixture of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), sphingomyelin, and dihydrocholesterol over a calcium oxalate subphase of relative supersaturation 5[12] and compressed to 32 mN/m, a pressure comparable to that of membrane bilayers.[13,14] In such monolayers, the sphingomyelin/dihydrocholesterol-rich regions form liquid-ordered (LO) domains (similar to LC domains of a single-component lipid phase diagram) surrounded by LE phase. The phase separation is easily observed with Brewster angle microscopy (BAM), as shown in Figure 1a. The rafts appear as light gray islands surrounded by the darker expanded phase. The sphingomyelin/dihydrocholesterol domains are initially circular and of size on the order of 10−20 μm (Figure 1a). The rafts merge to form larger domains, reaching sizes of several hundred micrometers within 30 min (Figure 1b).[15] This behavior is expected for LO domains and has been previously observed for lipid raft membrane models.[5] The lipid rafts that form at the air/water interface are similar to those characterized by AFM and fluorescence microscopy,[4−10] although the larger sizes observed here are likely due to the lack of monolayer support.

We have previously demonstrated that COM crystallization at phospholipid monolayers can be readily monitored with Brewster

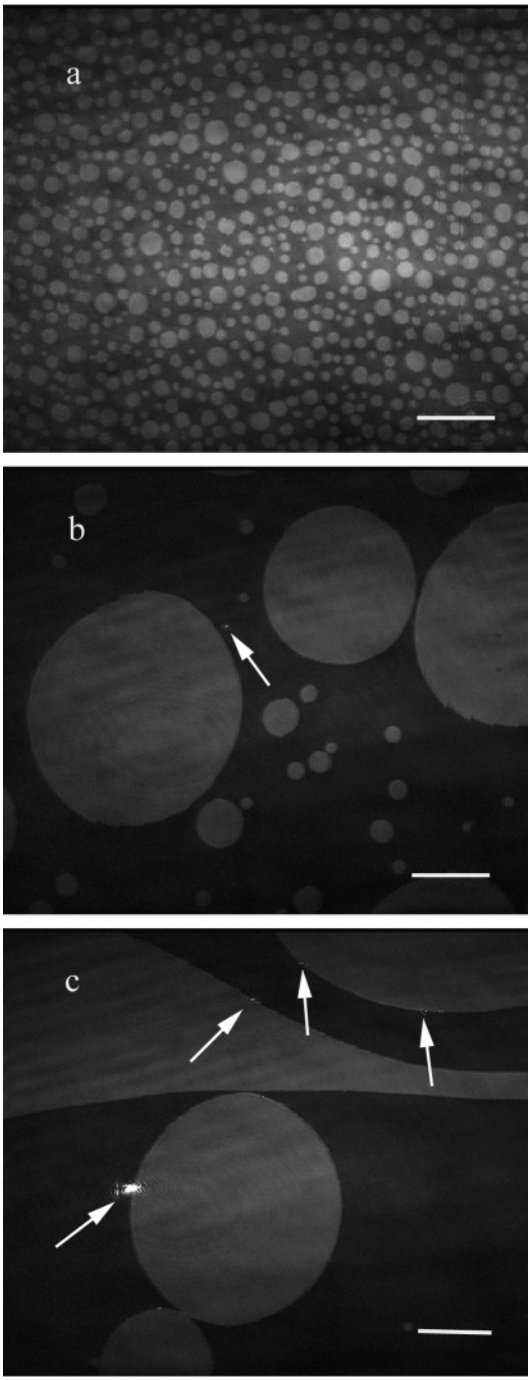

Figure 1. BAM images of a 2:1:1 POPC/SM/dihydrocholesterol monolayer at 32 mN/m over an RS 5 subphase at 25 °C after (a) 0 h (shutter timing, ST = 1/50 s), (b) 0.5 h (ST = 1/120 s), and (c) 3.5 h (ST = 1/120 s). The dark background is POPC, the light gray islands are SM/dihydrocholesterol, and the bright spots are COM. The arrows point at crystals precipitating at the expanded phase and phase boundaries. The scale bars represent 100 μm.

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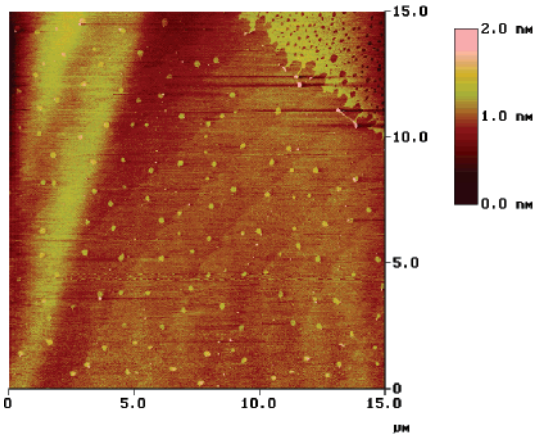

Figure 2. AFM image of a 2:1:1 POPC/SM/dihydrocholesterol film transferred on mica after being held for 1 h at 32 mN/m. The image is a 15 × 15 μm^2 scan showing the edge of a large LO domain and LO domains of submicrometer size.

angle microscopy.[3] Crystals appear as bright objects that are easily identified and quantified to assess the effects of different conditions on crystallization. Control experiments demonstrate that crystal formation is heterogeneous,[16−18] there is no bulk precipitation, and electron microscopy of transferred monolayers confirms the crystal habit of COM.[3,18] Figure 1b,c shows COM precipitation at the 2:1:1 POPC/sphingomyelin/dihydrocholesterol monolayer. In a period of 3 h, approximately 80% of COM crystals are observed at the LO phase boundaries (Figure 1c).

As viewed with BAM, the remaining 20% of crystals appear to form at the expanded phase (Figure 1b). The result is surprising in light of previous work with single-component and two-component lipid monolayers with LC/LE phase coexistence that showed COM precipitation predominately at phase boundaries or inside condensed domains.[3] However, the limited resolution of BAM leaves the possibility that crystals that appear at the expanded phase are actually at phase boundaries of LO domains too small (below 1 μm) to be detected by BAM. Therefore, an AFM image was taken of a 2:1:1 POPC/sphingomyelin/dihydrocholesterol monolayer that was held at 32 mN/m for 1 h and then transferred vertically to mica. Figure 2 shows the edge of a large LO domain. It is clear that, in addition to the large rafts, there are also submicrometer domains present. AFM images of a similar film transferred immediately after reaching 32 mN/m also have submicrometer rafts, suggesting that these small domains are present at all times. These features are consistent with similar observations on stearic acid monolayers transferred in the LC/LE coexistence region by Chi et al., who reported two types of condensed domains, large islands of several micrometers in size and grains with diameters of 20−130 nm.[19,20] It is very likely that even the 20% of COM identified as precipitating at the expanded phase by BAM is actually precipitating at the phase boundaries of small LO rafts.

We have suggested that the lipid phase boundaries are active sites that provide an additional free energy gain when the lipid molecules associate with a crystal face upon COM precipitation.[3] However, if the boundary is static, a kinetic barrier exists that limits the ability of the lipids to reorganize to accommodate the incipient crystal.[3] We have shown that, under conditions where two different lipids are phase separated so that the expanded and condensed phases are not in dynamic equilibrium, then crystals no longer form at the phase boundaries. With respect to the lipid rafts, the question that arises is whether the molecules of the raft domains are exchanging with the LE phase. The mean molecular area at 32 mN/m from compression isotherms for a mixture of sphingomyelin/dihydrocholesterol alone is 35.5 Å^2, and that for POPC alone is 83.6 Å^2 (isotherms not shown). For a 2:1:1 POPC/sphingomyelin/dihydrocholesterol monolayer, the POPC area (A_{POPC}) would be 2.4 times larger than that of the LO domains (A_{LO}) if the rafts contained no POPC. However, the measured A_{POPC}/A_{LO} ratio from BAM images is 1.4 ± 0.3, indicating that some POPC is incorporated in the rafts. The fact that POPC is at both expanded and ordered regions suggests that molecular exchange between phases is occurring. We can hypothesize that the dynamic phase boundary provides a site for densely packed molecules associated with the condensed LO phase to reorganize to optimally interact with the nucleating COM crystal.

In summary, we have prepared a Langmuir monolayer containing lipid rafts as a model for phase-separated domains in lipid membranes and demonstrated that heterogeneous precipitation of COM from metastable solution occurs at the monolayer. Furthermore, COM crystals form preferentially at phase boundaries between the LO lipid rafts and the expanded phase. The observations add support to the idea that membrane heterogeneities can act to catalyze calcium oxalate precipitation, of relevance to the problem of urinary stone formation.

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Supporting Information Available: All experimental details. This material is available free of charge via the Internet at htpp://pubs.acs.org.

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