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Lysophosphatidylcholine Reversibly Arrests Exocytosis and Viral Fusion at a Stage between Triggering and Membrane Merger*

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Little is known of the events occurring between membrane fusion triggering and subsequent fusion steps. To dissect this process we applied a reversible inhibitor of membrane fusion, lysophosphatidylcholine, to arrest exocytosis and virus-mediated syncytia formation. Next Ca^{2+} or H^+ (the respective fusion triggers) was administered and later removed. Then, inhibitor was withdrawn and fusion ensued, demonstrating that triggering causes the formation of an "activated state," which later develops into the fused state. Therefore, while different fusion processes utilize different triggers, the pivotal step involving membrane merger is trigger-independent and lipid-sensitive.

Triggered membrane fusion in cells involves membrane docking and subsequent membrane merger. Recent developments suggest conservation of docking mechanisms in disparate fusion reactions (1, 2). In contrast, the regulatory components of membrane fusion, which respond to different triggers, are most likely unique. It is not known if the mechanism of membrane merger is conserved in different fusion reactions. Calcium-triggered exocytosis and low pH-triggered viral fusion are diverse examples of cell biological processes that still share a common step, membrane fusion (3–5). In addition to using different triggers, they also differ in which leaflets of the fusing membranes make initial contact ("endoplasmic" *versus* "exoplasmic" fusion) (6, 7). Perhaps the most striking difference is speed; calcium-triggered exocytotic fusion, such as is seen in synaptic terminals, can be 1000 times faster than viral fusion (5). Based on these differences it was proposed that while these membrane rearrangements are superficially similar, they may have fundamentally different mechanisms (5). In particular, the rapidity of triggered exocytosis was hypothesized to reflect a situation where the introduction of calcium enabled a final single-step reaction, membrane fusion (5). In contrast, examples of viral fusion are known to involve not only triggering and membrane merger but also an intermediate commitment step (8) and the insertion of a hydrophobic viral peptide into the target membrane (9–11).

Ultimately, comparison of viral and exocytotic fusion mechanisms requires the identification of the fusion intermediates between triggering and membrane merger. Progress in this direction has been slow because of the short-lived nature of the intermediate structures and the absence of inhibitors to stabilize them. We recently found that the addition of exogenous lysolipids between contacting membranes inhibited fusion in four disparate biological processes, regardless of their particular trigger (calcium, pH, GTPγS,[1] or GTP) (12). This inhibition was not related to solubilization of requisite fusion components, irreversible denaturation, or membrane lysis (12). The apparently universal character of the phenomenon suggested that lysolipids inhibit an intermediate step common for all fusion reactions, fast and slow.

To determine which step of biological fusion is targeted by lysolipids and to test if examples of "slow" and "fast" fusion share common mechanistic steps, we studied two contrasting examples of triggered biological fusion. Low pH-triggered syncytia formation in baculovirus-infected cells (13, 14) is an example of slow fusion mediated by a known protein of the enveloped virus. The second system, the exocytotic fusion of sea urchin egg cortical granules, is a reaction that occurs in the eggs of most animals, including humans (15). This is an example of fast, calcium-triggered fusion, which can take place within an upper limit of 10–35 ms after the introduction of calcium (16). Because triggering occurs subsequent to docking in these systems, they are well suited for studying the final steps of membrane fusion.

EXPERIMENTAL PROCEDURES

Materials—All lipids and membrane dyes were purchased from Avanti Polar Lipids (Birmingham, AL) except for octadecyl rhodamine B (R18), which was purchased from Molecular Probes (Eugene, OR). Stock solutions of lysolipids were freshly prepared as a 0.5% (w/w) aqueous dispersion. Stock solutions of R18 (100 μM) and *N*-(lissamine rhodamine B sulfonyl) diacylphosphatidylethanolamine prepared from egg (Rhod-PE, 400 μg/ml) were made in ethanol.

Cortical Granule Exocytosis—Lytechinus pictus eggs were attached to polylysine-coated glass slides and then lysed with a jet of isotonic calcium-free buffer as previously described (17) except PKME buffer (50 mM Pipes, pH 6.7, 425 mM KCl, 10 mM $MgCl_2$, 5 mM EGTA, 1 mM benzamidine) was used instead of cortex medium. Cortices were mounted in a microscope perfusion chamber and perfused with GP buffer (1 M glycine, 50 mM Pipes, pH 6.7, 1 mM benzamidine) containing 1.2 mM DM-nitrophen ("caged calcium," Calbiochem) and 0.5 mM $CaCl_2$. In some experiments the calcium indicator Rhod-2 (10 μM, Molecular Probes) was included in the buffer to measure caged calcium release. The release of calcium bound to DM-nitrophen was accomplished by application of a brief pulse of UV light (360 nm) through the microscope epifluorescence port with an electric shutter. With this design only the cortices within the field of view were exposed to calcium, allowing multiple experiments on the same slide. Fusion was monitored using DIC optics enhanced with a digital video processor (LKH-9000, L. K. Hawke, Inc., Research Triangle Park, NC) on a modified Zeiss microscope (18). Calcium indicator fluorescence was measured using a photodiode attached to the microscope ocular with a 590-nm long pass filter. The signals were digitized by an ITC-16 computer interface (Instrutech Corp., Elmont, NY) and processed by commercial software (Igor; Wave-

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[1] The abbreviations used are: GTPγS, guanosine 5′-3-*O*-(thio)triphosphate; Pipes, 1,4-piperazinediethanesulfonic acid; DIC, differential interference contrast.