LIPID CELL BIOLOGY

Polyunsaturated phospholipids facilitate membrane deformation and fission by endocytic proteins

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Phospholipids (PLs) with polyunsaturated acyl chains are extremely abundant in a few specialized cellular organelles such as synaptic vesicles and photoreceptor discs, but their effect on membrane properties is poorly understood. Here, we found that polyunsaturated PLs increased the ability of dynamin and endophilin to deform and vesiculate synthetic membranes. When cells incorporated polyunsaturated fatty acids into PLs, the plasma membrane became more amenable to deformation by a pulling force and the rate of endocytosis was accelerated, in particular, under conditions in which cholesterol was limiting. Molecular dynamics simulations and biochemical measurements indicated that polyunsaturated PLs adapted their conformation to membrane curvature. Thus, by reducing the energetic cost of membrane bending and fission, polyunsaturated PLs may help to support rapid endocytosis.

Using electron microscopy, we analyzed our liposomes incubated with dynamin, endophilin, and GTP, a mixture optimal for membrane fission (12). Before incubation, the liposomes displayed a similar size distribution (radius $R \approx 30$ to 200 nm) (Fig. 1, D and E). After incubation, polyunsaturated liposomes were consumed into small vesicles ($R \approx 20$ nm), whereas monounsaturated liposomes appeared unchanged (Fig. 1, D and E, and fig. S2, A and D). When GTP hydrolysis was blocked, characteristic endophilin-dynamin spirals (8, 9) formed on polyunsaturated liposomes, but not on monounsaturated liposomes (Fig. 1D and fig. S2, B, C, and E). Thus, polyunsaturated membranes are sensitized to the mechanical activities of the endophilin-dynamin complex.

Adhesion of liposomes to the electron microscopy grid can lead to an overestimate of authentic fission owing to membrane breakage on the stiff support (23). To overcome this caveat, we used fission assays based on visualization of membrane PLs by fluorescence microscopy. First, we incubated GUVs with dynamin, endophilin, and GTP and monitored the GUV diameter over time (Fig. 1A). Because GTP hydrolysis occurs through contacts between dynamin molecules (11), GUV diameter measurements may reflect the rate of endocytosis. Using electron microscopy, we analyzed our liposomes incubated with dynamin, endophilin, and GTP, a mixture optimal for membrane fission (12). Before incubation, the liposomes displayed a similar size distribution (radius $R \approx 30$ to 200 nm) (Fig. 1, D and E). After incubation, polyunsaturated liposomes were consumed into small vesicles ($R \approx 20$ nm), whereas monounsaturated liposomes appeared unchanged (Fig. 1, D and E, and fig. S2, A and D). When GTP hydrolysis was blocked, characteristic endophilin-dynamin spirals (8, 9) formed on polyunsaturated liposomes, but not on monounsaturated liposomes (Fig. 1D and fig. S2, B, C, and E). Thus, polyunsaturated membranes are sensitized to the mechanical activities of the endophilin-dynamin complex.

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PLs remained unchanged (fig. S3 and movie S1). Second, we added dynamin, endophilin, and GTP to preformed membrane tubes to observe fission events directly (14). Again, the difference between mono and polyunsaturated PLs was notable: 95% of the tubes (n = 96) containing polyunsaturated PLs underwent fission, as compared with 4% of the tubes (n = 52) made of monounsaturated PLs (fig. I, F and G, and movie S2).

Next, we tested the effects of polyunsaturated PLs on the mechanical properties of the plasma membrane (PM), on which endophilin and dynamin act. We cultured epithelial cells (RPE1) with defined fatty acids and used optical tweezers to pull tubes from the PM. Lipid analysis showed that cells incorporated C22:6 into cellular PLs within a few hours (Fig. 2A and fig. S4). To facilitate mechanical manipulation, we cultured cells on L-shaped micropatterns on which they adopted a triangular shape (Fig. 2B). We pulled tubes from the cell hypotenuse and measured the pulling force (F) and the tube apparent radius (R) (Fig. 2, B to D, and fig. S5, A to C). Cells incubated with C22:6 showed a 1.8-fold decrease in F and a 2.5-fold decrease in R within 1 hour, whereas cells incubated with C18:1 showed no significant changes (Fig. 2, E and F). Because F and R are related to the bending rigidity (κ) and membrane tension (σ) by κ = FR/R^2 and σ = FR (15), the parallel decrease in F and R indicates that C22:6 incorporation decreased the bending rigidity by ~fourfold (Fig. 2G), as compared with a twofold decrease for GUVs containing 30 mol% polyunsaturated PLs (Fig. 2H), but did not affect membrane tension, which depended on interactions with the actin cytoskeleton (fig. S5, D to F).

By making the PM more flexible, polyunsaturated PLs might facilitate endocytic events—in particular, clathrin-mediated endocytosis—as suggested by the effect of C18:3, the precursor of C22:6, on model dopaminergic cells (16). Cells fed with C22:6 displayed a 1.5-fold higher transferin (Tfn) uptake than C18:1-fed cells (Fig. 3, A and B). Because cholesterol alleviates membrane deformation stress by flip-flopping between bilayer leaflets (17–19), we depleted cholesterol from the PM using methyl-β-cyclodextrin (MCD) to better isolate the contribution of polyunsaturated PLs. MCD completely inhibited Tfn uptake in C18:1-fed cells, but not in C22:6-fed cells (Fig. 3, A and B). Moreover, when we overexpressed endophilin, Tfn uptake under low-cholesterol conditions was nine times more efficient in C22:6 than in C18:1-treated cells (Fig. 3, A and C). Dyno 4a, a specific dynamin inhibitor (20), inhibited this endophilin-stimulated endocytosis (fig. S6).

Thus, polyunsaturated PLs can facilitate endocytic events that are under the dual control of endophilin and dynamin. Polyunsaturated acyl chains reside closer to the bilayer-water interface than other acyl chains, owing to the ability of polyunsaturated acyl chains to adopt bent conformations (21, 22). How this feature varies with curvature should give insight into the benefit of polyunsaturated PLs for membrane deformation. However, given the difficulties in addressing lipid conformation in membranes...
of complex geometries, we performed coarse-grain molecular dynamics (MD) simulations. We mimicked the process of membrane deformation by applying a pulling force to a phosphatidylcholine (PC) bilayer (23). The resulting membrane tube was thinner when the bilayer contained polyunsaturated PLs compared with monounsaturated ones, suggesting a decrease in bending rigidity (Fig. 4B and movie S3).

Estimating the bending modulus from buckled bilayers (fig. S7A) or from the undulations of flat bilayers (Fig. 4B and movie S3) indicated a twofold decrease in \( k \). Next, we increased the pulling force and observed that the tube frequently underwent fission (Fig. 4A and movie S3). Computing the force and tube radius immediately before fission indicated that polyunsaturated PLs lowered the force threshold (Fig. 4B and fig. S7C) but did not change the critical radius for fission (\( R_c \approx 4 \text{ nm} \)). Thus, the coarse-grain simulation approach recapitulates the properties of polyunsaturated membranes: they are more prone to deformation and fission.

We therefore used MD to analyze the conformation of the PL acyl chains. In agreement with previous studies (24), the bending angle (\( \theta \)) of the polyunsaturated acyl chain showed several

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**Fig. 2. Incorporation of C22:6 into cellular PLs decreases PM bending rigidity.** (A) Mass spectroscopy analysis of phosphatidylcholine species in RPE1 cells after incubation with C22:6. (B) Differential interference contrast and fluorescence confocal image of a RPE1 cell plated on an L-shaped micropattern. A tube was pulled from the PM using a 3-μm concavalin A-coated bead and an optical tweezer. Scale bar, 10 μm. (C and D) Typical measurements of the force (\( F \)) and apparent radius (\( R \)) of cell tubes after incubation with C18:1 or C22:6 fatty acid for 1 hour. <\( F_0 \)> and <\( R_0 \)> are the mean force and mean radius, respectively, before fatty acid addition. W/O, without; OA, oleic acid; DHA, docosahexaenoic acid. Scale bar, 5 μm. (E and F) Evolution of \( F \) and \( R \) over time after incubation of the cell culture with C18:1 or C22:6 fatty acids (73 tubes in four independent experiments with C22:6 and 71 tubes in four independent experiments with C18:1). Error bars denote the SD of each mean. (G) Evolution of the bending modulus (\( k \)) and tension (\( \sigma \)) as deduced from (E) and (F). Error bars denote the SD of each mean. (H) Tube-pulling experiments on GUVs containing 30 mol % C16:0-C18:1 PE or C18:0-C22:6 PE (\( n = 7 \) GUVs for each condition). A linear fit of \( F^2 \) versus \( \sigma \) gives \( k = (15 \pm 3)k_B T \) for C16:0-C18:1 PE and \( (7 \pm 3)k_B T \) for C18:0-C22:6 PE (\( k_B \), Boltzmann constant; \( T \), temperature). Scale bar, 10 μm.
Fig. 3. Polyunsaturated PLs stimulate Tfn endocytosis. (A) RPE1 cells overexpressing or not expressing endophilin A1 were cultured in the presence of BSA-C18:1 or BSA-C22:6 complexes for 3 hours and, when indicated, were further treated with MCD. At time = 0, fluorescent Tfn was added, and the amount of internalized Tfn at 37°C after 5 min was determined by confocal microscopy. Data were analyzed using a Student’s t test with Welch correction: ***P < 0.0001 (≈450 cells for each condition). n.s., not significant. (B) Confocal images of total (internalized + PM) Tfn in cells incubated with C18:1 or C22:6 fatty acids and treated or not treated with MCD before endocytosis. (C) Images of internalized Tfn in C18:1- or C22:6-fed cells overexpressing endophilin A1 and, when indicated, treated with MCD before Tfn endocytosis. Colored images: single confocal planes of internalized Tfn (red) and PM (green). Black and white images: z-stacked projection of Tfn fluorescence. Scale bars (B and C), 10 μm.

Fig. 4. Polyunsaturated PLs adapt their conformation to membrane curvature. (A) Coarse grain simulation of a PC bilayer subjected to a pulling force: a tube forms and eventually undergoes fission. R, radius. (B) Tube radius versus pulling force for bilayers containing mono or polyunsaturated PC. The dashed line indicates the critical radius for fission. Error bars indicate the SD of each mean. (C) Distribution of the bending angle (θ) of the mono- or polyunsaturated acyl chain in flat or spherical (R = 15 nm) bilayers. (D) Characteristic area constant of deep and shallow lipid-packing defects in bilayers of the indicated shape and containing 0 or 30 mol % polyunsaturated PC. (E) Models showing that polyunsaturated PLs adapt their conformation to membrane curvature, thereby favoring the formation of shallow lipid-packing defects at the expense of deep ones.
peaks (Fig. 4C). Positive membrane curvature shifted the angular distribution toward the kinched conformations (40°, 80°) at the expense of the more extended one (130°), whereas the angular distribution of the monounsaturated acyl chain, which peaked at θ = 130°, was almost unaffected (Fig. 4C).

We then looked for defects in the geometrical arrangement of lipids by scanning the surface of simulated bilayers of various shapes (Fig. 4D). A defect is a region where the first lipid atom encountered by a line normal to the surface is an aliphatic carbon (25). Using a depth threshold of 1 Å below the glycerol region (fig. S8, A and B), we observed that both deep and shallow defects increased with positive membrane curvature (Fig. 4D). However, polyunsaturated PLs promoted the formation of shallow defects and decreased the formation of deep defects, especially in curved membranes where defects were abundant (Fig. 4D and fig. S8, C and D). Thus, the angle and packing defect analyses agreed: Polyunsaturated PLs adapt their conformation to membrane curvature by using their flexible chain to fill voids in the outer monolayer (Fig. 4E).

Polyunsaturated PLs are beneficial for health and their abundance in the brain suggests a decisive advantage for cognitive functions, but the underlying molecular mechanisms are poorly understood. By showing that polyunsaturated PLs improve the response of model membranes to the mechanical activities of endocytic proteins, our study offers a potential explanation for extraordinary speed of endocytosis in the nerve terminal (26), where polyunsaturated PLs are abundant (29). The conformational plasticity of polyunsaturated PLs may also explain their role in mechanotransduction (50).

REFERENCES AND NOTES

SUPPLEMENTARY MATERIALS
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Materials and Methods
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