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Elasticity-Driven Membrane Budding through Cholesterol Concentration on Supported Lipid Monolayer–Bilayer Junction

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Membrane budding is an essential process during various biological activities, such as intercellular communication and transportation of life-sustaining molecules and signals, which primarily occur with the aid of particular proteins. During such processes, the presence of cholesterol and its assembly into particular domains within the membranes have attracted considerable attention due to their unique characteristics in terms of the regulation of signal activities, membrane fluidity, and hormone production during metabolic pathways. However, despite these crucial roles, the precise mechanisms of their spatiotemporal localization toward specific areas and their physiological roles in membrane budding without the assistance of particular proteins remain unclear. Herein, a model membrane platform is reconstituted with lipid monolayer–bilayer junctions, which facilitate the selective concentration of cholesterol on the lipid monolayer regions. In vitro membrane budding is demonstrated by the remaining vesicles where the lipid monolayer membrane is ruptured. The physicochemical approach to the selective concentration of cholesterol and its consequent membrane vesiculation offer important clues to help understand the underlying mechanisms of cholesterol in the lipid-driven membrane-budding process.

Membrane budding, an essential step in structural and compositional changes of membrane constituents and their distribution, is a vital cellular function involved in synaptic activity regulation,[1] vesicle transport,[2] multivesicle body biogenesis and release of enveloped viruses,[3] vesicle fusion,[4] and endocytosis/exocytosis.[5] These conformational changes arise from 1) the inhomogeneous distribution of lipids in different molecular shapes;[6] 2) curvature-scaffolding proteins (e.g., BAR domain proteins, clathrin, and COPII proteins);[7] and 3) vertical alignments of embedded proteins or even cytoskeletal elements in lipid bilayers.[4]

As one of a variety of lipids, a cholesterol has attracted considerable attention due to its unique molecular properties, such as high curvature,[8] ring-shaped molecular structure, and high elastic energy when coalesced.[9] From a physiological perspective, cholesterol facilitates "vesicle fusion/docking" and synaptic vesicle trafficking. The high molecular ratio (>40 mol%) of cholesterol in neuron vesicles[1,10] and drastic inhabitation of the vesicle docking and trafficking by the cholesterol deprivation[5,11] imply its significant functions in neuronal activities. Also, recent findings that extracellular vesicles (diameter, d < 200 nm) from the plasma membrane comprise a large concentration of cholesterol[12] hints the critical roles of cholesterols during a neurotransmitter release and membrane endocytic/exocytic budding processes.[13] However, the precise mechanisms of their spatiotemporal localization toward a specific area and physiological roles in membrane budding without the assistance of particular proteins[14] and underlying mechanism of the budding processes by cholesterol have not yet been fully elucidated.

As a model membrane system in vitro, a supported lipid membrane (SLM) provides a biological membrane interfaced with a solid surface for medical diagnostics and sensor applications.[9,15] The SLM was achieved by exposing small unilamellar vesicles (SUVs; d < 100 nm) to a solid support to form a lipid sheet from the spontaneous vesicle rupture.[16] However, for reconstructing cholesterol-enriched SLMs, it remains challenging to prepare the SUVs that contain cholesterol over 30 mol%.[17] This is attributed to the distinct physical properties of cholesterol-rich SUVs when compared to low-cholesterol SUVs, including such high transition temperature, large bending rigidity,[18] and well-organized phases, which interfere with spontaneous vesicle rupture due to their enhanced energy barrier.[19] Specialized strategies to reconstruct the SLM in high concentrations of cholesterol have been introduced with the aid of membrane-active peptide addition,[20] hydrodynamic force, or solvent-assisted lipid self-assembly.[17] However, the SLM with cholesterols and related unique biological phenomena still remain mysterious.

Here, we report a customized set of criteria to reconstitute SLMs for selective concentrations of cholesterol-enriched domains in vitro. A connective lipid monolayer–bilayer

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