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Lamellar Sheet Exfoliation of Single Lipid Vesicles by a Membrane-Active Peptide

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Materials and Methods

Vesicles were prepared by the extrusion method. Briefly, a chloroform solution of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[biotinyl(polyethylene glycol)-2000 (DSPE-PEG(2000)biotin) (0.1 mol %), and1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl (rhodamine-PE) 0.5 wt % was first dried using a flow of nitrogen (all lipids purchased from Avanti Polar Lipids). The dried lipid film was kept under vacuum for at least 3 h to remove traces of chloroform. The lipid film was rehydrated with tris buffer (pH 7.5) containing 100 mM NaCl, 10 mM tris and 10 mM calcein. After vortex mixing of the solution of hydrated lipids (4 mg/mL), vesicles of different size were made by a Mini Extruder (Avanti Polar Lipids) using polycarbonate membrane with a pore size of 200 or 50 nm. Free calcein was removed by a Sephadex G-25 gel filtration column. The average vesicle diameters were measured by the dynamic light scattering (DLS) technique. The AH peptide (H-Ser-Gly-Ser-Trp-Leu-Arg-Asp-Val-Trp-Asp-Trp-Ile-Cys-Thr-Val-Leu-Thr-Asp-Phe-Lys-Thr-Trp-Leu-Gln-Ser-Lys-Leu-Asp-Tyr-Lys-Asp-NH_2) was synthesized by Anaspec Corp. (San Jose, CA).

TIRF-based Single Vesicle Assay

Glass microscope coverslips were first cleaned by SDS (1%) and treated by oxygen plasma (Plasma Cleaner PDC-32G, 18W, Harrick Plasma USA) for 1 min and then assembled into commercial sticky-slide VI 0.4 microfluidic chambers (ibidi GmbH, Martinsried, Germany). The coverslips were coated by self-assembled monolayer of a 10000:1 mixture of poly(L-lysine)-grafted poly(ethylene glycol) (PLL-g-PEG) and PLL-g-PEG(-biotin) (SuSoS AG). The surface was further incubated with neutravidin (10 μg mL^{-1}) for 30 min and then rinsed with buffer carefully. Biotinylated POPC vesicles loaded with calcein were immobilized at concentration of 0.1 μg mL^{-1} for 15 min. The AH peptide (100 nM) was injected using a peristaltic pump at a flow rate of 100 μL/min. Vesicles were imaged using total internal reflection fluorescence (TIRF) microscopy. Using alternating emission filters (FITC and TRITC), time-lapse imaging was applied to record the fluorescence emission from calcein and rhodamine upon addition of AH peptide.

TIRF Microscopy Setup

TIRF microscopy was performed on an inverted Eclipse TE 2000 microscope (Nikon) equipped with a high-pressure mercury lamp, an Apo TIRF 60× oil objective (NA 1.49), and a Luca EMCCD camera (1002 × 1004 pixel). Filter sets and dichroic mirrors in the filter cubes were chosen to match the excitation and emission properties of the selected fluorophores.

Single Vesicle Data Analysis

Image analysis was conducted in Matlab 2010. A vesicle was defined as a group of at least three connected pixels exceeding an intensity threshold set at 3–6 times higher than the average noise level. The intensity time trace of each vesicle was obtained by pixelwise integration and subsequent background correction over the complete time series. Subsequently, these time traces were fitted by a piecewise defined function composed of a linear decay during the first part (lag period) and a monoexponential decay during the second part (contents release period). A least-squares fitting method gave access to the two decay rate constants, the initial vesicle intensity, and the point in time when the transition between linear and monoexponential decay takes place, i.e., the onset of contents release or vesicle rupture.