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Report:

We conducted a high hydrostatic pressure X-ray reflectivity (XRR) study on the insertion mechanism of fusogenic peptides of viral envelope proteins into solid-supported model membranes. Enveloped viruses enter host cells via protein-mediated membrane fusion. Fusion proteins embedded in the viral envelope interact with the cell membrane and pull cell membrane and viral envelope towards each other. Our study focused on the role of fusion peptides (FP) and transmembrane domains (TMD) of viral fusion proteins in the membrane fusion process. FPs are located in the ectodomain and their insertion into the target membrane leads to destabilization and, thus, catalyses the fusion reaction. TMDs anchor the fusion proteins in the viral envelope and play an important role in the stabilization of fusion pores at a later stage of membrane fusion. We prepared solid-supported DMPC (1,2-Dimyristoyl-sn-glycero-3-phosphocholine) bilayers on silicon wafers by spin-coating [1,2]. The wafers were placed in a custom-made high hydrostatic pressure cell [3] ($p_{max} = 5000\ bar$) immersed in BisTris buffer solution at fusogenic pH 5 containing the investigated peptide. At the applied sample temperature of 45 °C the solid-supported DMPC model membranes are in the liquid-crystalline L_α phase at low pressures. When the pressure is increased, a phase transition into a gel-like state occurs at around 750 bar so that we were able to study the membrane-peptide interactions in both phases. We applied a photon energy of 70 keV and a beam size of approximately 3µm (vertical) × 40 µm (horizontal).

Some of our results concerning the TMD of the vesicular stomatitis virus (VSV) are presented in the figures below. The first one shows the reflectivity data of DMPC bilayers at 50 and 2500 bar in absence and presence of VSV-TMD. In both cases the phase transition is reflected in a shift of the occurring oscillations to lower angles. Information on structural details is provided by the vertical electron density profiles in the next diagram. The laterally averaged electron density ρ is shown as a function of the distance z to the wafer surface. It can be seen that VSV-TMD increases the electron density especially in the hydrophobic area in the L_α phase at 50 bar, while it decreases the electron density at 2500 bar. Presumably, the peptide reduces the order in the gel-like phase so that the tail groups are less densely packed. The last figure shows the phase transition of solid-supported DMPC bilayers reflected in their thickness d as a function of the pressure p. We found that the addition of the peptides has only minor influence on the phase boundary. With the applied pressure increments, only a slight shift to lower pressures can be assumed.

Further measurements showed that the penetration of fusogenic peptides in DMPC bilayers can also lead to a displacement of lipids into an additional bilayer, indicated by a doubling of the layer thickness. The evaluation of this observation and further experimental data is still in progress.

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References:

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