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Report:

We conducted a combined grazing incidence diffraction (GID) and X-ray reflectivity (XRR) study on the interaction of fusogenic peptides of viral envelope proteins with anionic lipid monolayers at the air-water interface. 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 spread the phospholipid 1,2-Dipalmitoyl-sn-glycero-3-phosphatidic acid (DPPA) on the surface of a phosphate buffer subphase at pH 5 in a Langmuir trough to obtain a well-arranged monolayer. The fusion peptides were dissolved in 1 ml buffer and then injected underneath the monolayer, resulting in a final peptide concentration of 2 µM. We studied four different fusogenic peptides. The class I fusion peptide of Hemagglutinin 2 (HA2-FP), the class II fusion peptide of Tick-borne encephalitis virus (TBEV-FP), the class III fusion peptide of vesicular stomatitis virus (VSV-FP) and the transmembrane domain of VSV (VSV-TMD). The peptides were added at surface pressures of the DPPA film of 15, 25, and 35 mN/m. XRR and GID scans were performed before and after the injection at a photon energy of 22 keV. During the experiments, the sample environment was flushed with helium in order to suppress air scattering and to prevent oxidative beam damage. The DPPA lipids form a two dimensional hexagonal lattice with their hydrocarbon chains oriented almost perpendicular to the water surface at all considered surface pressures. Therefore, only one Bragg reflection occurs in the GID patterns at $q_z = 0$. Figure 1 and 2 show this Bragg reflection for DPPA films at 15 and 35 mN/m before and after the injection of HA2-FP as a function of $q_{||}$. It can be seen that the reflection shifted to higher $q_{||}$ and became sharper after the addition of HA2-FP at 15 mN/m, while it remained almost constant at 35 mN/m. From the positions and widths of the reflections, the lattice constants a and crystallite sizes L of the lipid monolayers can be determined. Figure 3 shows how the lattice constant changed after the injection of HA2-FP as a function of time. We observed that HA2-FP compressed the film at 15 and 25 mN/m and reduced

a close to the value of DPPA at 35 mN/m. At 35 mN/m, HA2-FP had no effect on the lattice constant within the experimental resolution. However, the observed compression at the lower surface pressures suggests that HA2-FP penetrated the monolayers and displaced the lipids. Figure 4 shows that the crystallite size was almost constant or increased slightly during this process, indicating that the HA2-FPs formed clusters at the surface and did not spread evenly over the film. By simulating the reflectivity curves of HA2-FP interacting with a DPPA monolayer at 15 mN/m in figure 5, we obtained the vertical electron density profiles in figure 6. We observed that the peptides accumulated underneath the lipid head groups from an increase of the electron density in this region. Their insertion into the monolayer led to a reduction of the electron density of the lipid film.

The other peptides show a very different behavior. While VSV-FP compressed the monolayer to a much lesser degree than HA2-FP, the addition of TBEV-FP slightly increased the lattice constant and caused an increase of the electron density in the lipid tail group region. VSV-TMD barely affected the vertical structure of the lipid film but reduced the crystallite size. The evaluation of the data is still in progress. A detailed comparison of the behavior of the different fusogenic peptides will contribute to the understanding of viral cell fusion on a molecular level.

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