Experimental report

07/10/2024

Proposal: 8-02-997 **Council:** 4/2023

Title: SARS CoV-2 Spike protein is remodelling HDL. What are the structural consequences of this building usingdifferent HDL lipid compositions?

Research area: Chemistry

This proposal is a new proposal

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Samples: dDMPC
DMPC, POPC, PLPC, Cholesterol, ApoA1
SARS Cov2 spike protein

Instrument	Requested days	Allocated days	From	To
D17	4	0		
FIGARO	4	4	06/11/2023	10/11/2023

Abstract:

SARS-CoV-2 Spike (S) protein binds cholesterol and high density lipid protein (HDL) and affects the function of HDL (incl. ability to exchange lipids). Indeed, thanks to neutron reflection experiments, we found out that both, the model donor membrane¿s composition as well as the presence of the S protein affects the HDL¿s capacity to deposit and exchange lipids with a model membrane. Moreover, the S protein takes preferably DMPC and PLPC when in presence of cholesterol and is not uptaking POPC. We now want to verify that the function of HDL is affected through the selective removal of lipids from HDL by S protein. Hence, we plan to examine the interaction of HDL with different lipid compositions with a model membrane when incubated with and without the S protein. Thereby we will use reconstructed (-r) HDL made of DMPC, PLPC or POPC and an deuterated DMPC model membrane, since we know the S protein does not removes saturated lipids such as DMPC without cholesterol at all. We expect to observe no effect of the S protein on the lipid exchange ability of r-HDL made of POPC, while a modulation of the r-HDL function should be expected for particles containing DMPC and PLPC.

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Instrument: Figaro
Schedule: 6/11/23 – 10/11/23

We aimed to measure how the remodeling of HDL upon incubation with SARS Cov-2 Spike protein affects the Interaction between HDL subfractions and a SLB, using tail deuterated DMPC (dDMPC). Unfortunately, the spike protein we produced for the experiment showed no to little activity leading us to adapt the experimental plan as follows:
We focused on measuring the interaction between the HDL total, and the subfractions HDL 3, HDL 2a and HDL 2b for a healthy normolipidemic individual as well as on our second set of experiments on a new project dealing with an alternative method to form SLBs. This method is known the Solvent exchange method, established for QCMD by Ferhan et al, 2019. However, the structure of the SLB has never been quantified not it is known whether any organic solvent remains attached to the bilayer. The presence of organic solvent in the SLB could potentially affect any subsequent interaction of, for example, a protein and the SLB.
In the solvent exchange method, the lipids get dissolved in an organic solvent (Ethanol or Isopropanol). The solid-liquid cell gets flushed by hand with the organic solvent to be fully filled with it and then the lipids are injected followed by a slow rinse with MQ H_2O. Then the SLB is measured in 3 contrasts: D_2O, cmSi and H_2O.
The critical points determining if the SLB forms or not are the Lipid concentration and the speed of the water rinse. We used POPC at a concentration of 0.5 mg/mL 0.75 mg/mL and 1 mg/mL in Isopropanol to determine an adequate lipid concentration. The flow rate was kept constant at 0.1 ml/min, which worked well with optimal concentration for best coverage at 0.75 mg/mL.
We also tried POPC 1mg/mL in Ethanol and got a multilayer instead.
We tried washing the mounted cells with Isopropanol to clean them from the SLB prior to depositing a new one. But that attempt failed twice, as the cells didn´t get fully clean again. This means that the same block cannot be re-used, but the cells have to be unmounted and cleaned properly including ozone treatment.
Finally, we formed a SLB using dDMPC at 0.75mg/mL in Isopropanol. We also tested dDMPC +40mol% Cholesterol mix (0.75mg/mL) in isopropanol which gave a multilayer.
We made kinetics measurements while rinsing the with MQ H_2O, finding out that the SLB is formed in at roughly 4 minutes into the rinsing step (4 mL, or roughly 2 times the solid liquid cell volume). However, we continued rinsing until the total of 70 min to determine if the remaining orgarnic solvent in the SLB could be washed out.
These experiments show the high dependency on the lipid concentration, lipid type and solvent used for the success of this approach to form SLB.
We compare the result of the dDMPC done by solvent exchange to the dDMPC SLBs done by vesicle fusion for the HDL interactions.
The three contrast data fitting for the dDMPC SLB enables determining the extent of organics solvent remaining within the bilayer core. We plan further measurements to evaluate weather the interaction of this organic solvent containing SLBs with Melittin, an antibacterial peptide, is comparable between an SLB done by vesicle fusion.