Experimental report

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Proposal: 8-02-943 **Council:** 10/2020

Title: Investigation of interaction between S2 subunit of SARS-CoV-2 Spike protein with model membranes composed of natural lipids

Research area: Biology

This proposal is a new proposal

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Samples: PROTEIN
LIPIDS

Instrument	Requested days	Allocated days	From	To
FIGARO	3	2	11/06/2021	13/06/2021

Abstract:

COVID-19 is still a poorly understood infectious disease, caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Cell entry is mediated by the viral Spike Protein, which is composed of two subunits: S1 and S2. Our previous NR research focused on peptides derived from S2 that were responsible for fusion of the viral and human cell membrane during infection. However, since the Spike protein is a trimer, these experiments may not have captured any cooperative process due to the concurrent involvement of multiple S2 fusion peptides.

We will therefore employ NR to investigate the membrane binding activity of the trimeric Fusion Core of the Spike S2 domain, upon its interaction with natural lipid monolayers and bilayers. The resultant data will be used to build molecular models of the Spike Protein S2 subunit bound to a planar membrane, and will therefore help clarify how lipid composition and environmental factors (such as pH) modulate Spike protein function.

Experimental report of #8-02-943 on FIGARO

Investigation of interaction between S2 subunit of SARS-CoV-2 Spike protein with model membranes composed of natural lipids

Scientific Background

β-Coronaviruses (CoVs) are single-stranded positive sense RNA viruses[1] that belong to class I viral fusion viruses. They can infect cells via direct membrane fusion mechanism[2], [3]. Upon viral entry, copies of the genome are made in the cytoplasm, followed by expression of viral proteins and final assembly of functional viral particles, which are then released from the cell. The main structural components of β-coronaviruses include a lipid envelope, the Spike (S), Membrane (M), and Envelope (E) proteins, as well as the Nucleoprotein (N), which forms complexes with the viral RNA. Although it is already well-know that the S1 spike protein domain contains the receptor binding site for the angiotensin-converting enzyme 2, ACE-II[4], it has to be underlined that the S2 spike subunit contains a fusion domain that is responsible for triggering the fusion between lipid bilayers. After S1-ACE-II binding, a proteolysis-triggered conformational change in the C-terminal S2 subunit occurs[4]: S2 continues to be embedded in the viral membrane, but its heptad repeat (HR) 1 and 2 domains associate to form a six-helix bundle fusion core[5]. Proteolysis at the S2' site (at residue 816) subsequently frees the Spike protein fusion domain, which associates with the host cell and initiates membrane fusion. However, the molecular mechanisms driving this fusion process are not deeply understood. The aims of the experiments summed up here were to unravel the fusion mechanism, by simplifying the systems down to its core elements: 22 to 25 amino acids peptides derived from the fusion domain of the Spike (called fusion peptides, FP1, 2 and 4) were employed, and their interaction with plasma membrane (PM) mimicking lipid monolayer was investigated.

Results

The results obtained from this beamtime have been included in Andreas Santamaria's PhD thesis, and is being readied for publication.

The full S2 subunit was not available, so instead we used peptides derived from it (fusion peptides, FP1, FP2 and FP4). Their interaction with model membrane composed of natural lipids and synthetic lipids were investigated at the air/water interface, by exploiting the low-Q_z approach (from 0.01 to 0.03 $Å^{-1}$). This approach directly provides the amount of material present at the interface, by using different lipid isotopic contrasts (*i.e.*, hydrogenous and perdeuterated lipids in the case of PM; hydrogenous phospholipids-deuterated cholesterol, deuterated phospholipids-hydrogenous cholesterol and deuterated phospholipids-deuterated cholesterol for the synthetic mixture). The PM model was made of natural phospholipids extracted from yeast grown at the ILL D-Lab, and purified at the lipid platform of ILL (L-Lab). Deuterated cholesterol was provided by the ANSTO deuteration platform. The synthetic lipid model was composed of POPC:POPS:cholesterol 3:1:1 molar ratio. Both compositions were previously exploited for studies of FPs-membranes interaction[6]–[8].

To investigate the interaction of FP1, FP2 and FP4 with lipid monolayer at different values of surface pressure (Π), PM lipid composition in chloroform containing 5% in mol FPs were employed, *i.e.*, a ratio FP:lipid of 1:20. This methodology allows depositing FPs-embedded monolayers in which the peptide partitions with the lipids, permitting to evaluate the composition of the film present at the air/water interface at any wanted surface pressure. We calculated the molar % of each species, lipids and peptides (see **Figure 1 A**): FP4 is the most abundant at any investigated surface pressure; then FP1 is found with a higher molar fraction than FP2, which is the only one whose molar fraction reaches zero at high pressures (Π=30 $mN{\cdot}m^{-1}$). These data are in agreement with previously published data that showed the binding efficiency of each peptide to the PM model membrane monolayer[8]. The case of FP2 is particularly interesting. Indeed, it has a net charge of zero, and it is the only one whose concentration in the monolayer tends to zero at high values of surface pressure. We exploited the synthetic POPC:POPS:cholesterol composition with 5% in

mol FP2 and we determined the molar fraction of phospholipids, cholesterol and FP2 at different surface pressures. The outcomes showed that the presence of the peptides produces a reduction in the amount of cholesterol partitioning at the interface, with respect to the one present in the purely lipid monolayer (see full symbols with respect to empty ones in **Figure 1 B**).

All the experiments were performed at physiological pH.

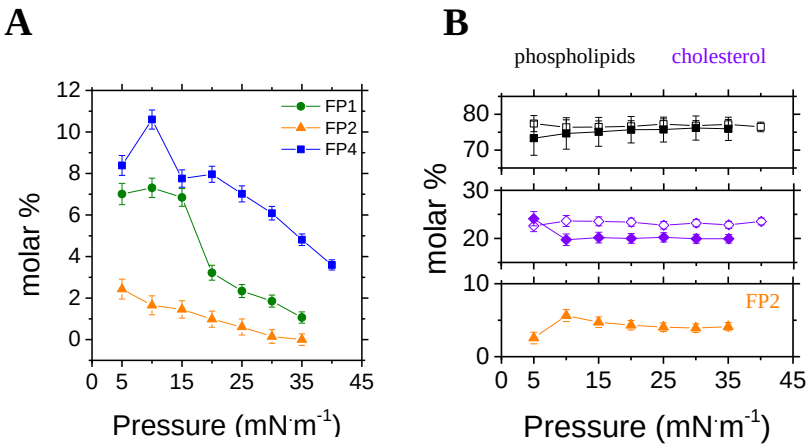

Figure 1 A Molar percentage of FP1 (green circles), FP2 (orange triangles) and FP4 (blue squares) obtained from low-Q_z measurement. **B** Data related to phospholipids (POPC and POPS), cholesterol and FP2 are shown as black squares, violet diamonds and orange triangles, respectively. Phospholipids and cholesterol data related to the purely lipids monolayer are shown as empty symbols.

References

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