Experimental report

15/01/2024

Proposal: 9-13-980 **Council:** 10/2020

Title: Influence of lipid-synthesizing enzymes on lipid transfer between membranes.

Research area: Biology

This proposal is a new proposal

Main proposer: Morgane MICHAUD

Experimental team: Morgane MICHAUD

Local contacts: Lionel PORCAR

Samples: Mixture of dPOPC + CDP-DAG liposomes
Mixture of dPOPC + CDP-DAG + Cho1 liposomes + Osh6
Mixture of dPOPC + CDP-DAG + Cho1 liposomes

Instrument	Requested days	Allocated days	From	To
D22	2	2	12/02/2021	13/02/2021

Abstract:

Non-vesicular lipid transport is an essential process to ensure membrane biogenesis and organelle function. In cells, the mechanisms involved in non-vesicular lipid transport is still poorly understood. Some lipid transfer proteins (LTPs) have been identified and the enzymes that synthesize lipids are suspected to play a role in this process. The objective of our study is to investigate whether Cho1, a yeast phosphatidylserine (PS) synthase, by itself and/or in concert with the LTP Osh6, is involved in the transfer of newly synthesized PS between membranes by using time-resolved small angle neutron scattering (TR-SANS). To do so, in vitro transport kinetics of newly synthesized hydrogenated PS (H-PS) from a deuterated (D) donor liposome to an acceptor D-liposome will be analyzed in different liposome reaction mixtures (Cho1 alone, Cho1 + Osh6 in different molecular ratios). From the decay curves, we will determine and compare essential transport parameters, like the transfer exchange constant or the half time of lipid transport for each condition. We expect to demonstrate that the active synthesis of lipid, in concert with LTPs, plays a key role in non-vesicular lipid transfer.

Report for experiment 9-13-980

Influence of lipid-synthesizing enzymes on lipid transfer between membranes.

Non-vesicular lipid transport is an essential process to ensure membrane biogenesis and organelle function. In cells, the mechanisms involved in non-vesicular lipid transport are still poorly understood. Some lipid transfer proteins (LTPs) have been identified and the enzymes that synthesized lipids are suspected to play a role in this process. The objective of our study was to investigate whether Cho1, a yeast phosphatidylserine (PS) synthase, by itself and/or in concert with the LTP Osh6, can promote the transfer of newly synthesized PS between membranes by using time-resolved small angle neutron scattering (TR-SANS) on D22. TR-SANS combined with contrast match (CM) have been previously used to study spontaneous lipid transfer between donor and acceptor liposomes, but never lipid transport mediated by lipid transfer proteins. The strategy planned was to analyze in vitro the transport kinetic of newly synthesized hydrogenated PS (H-PS) from a deuterated (D) donor liposome to an acceptor D-liposome in different liposome reaction mixtures. From the variation of the scattering intensity curves, it was planned to determine and compare essential transport parameters, such as the transfer exchange constant or the half time of lipid transport for each condition. We expected to demonstrate that the active synthesis of lipids, in concert with LTPs, played a key role in non-vesicular lipid transfer. 48h was allocated on D22 in order to 1) set up the lipid transport system with Osh6 alone (first session of 24h) and then to 2) analyze the transport mediated by Osh6 in presence of Cho1 in the donor liposome (proteoliposomes, second session of 24h).

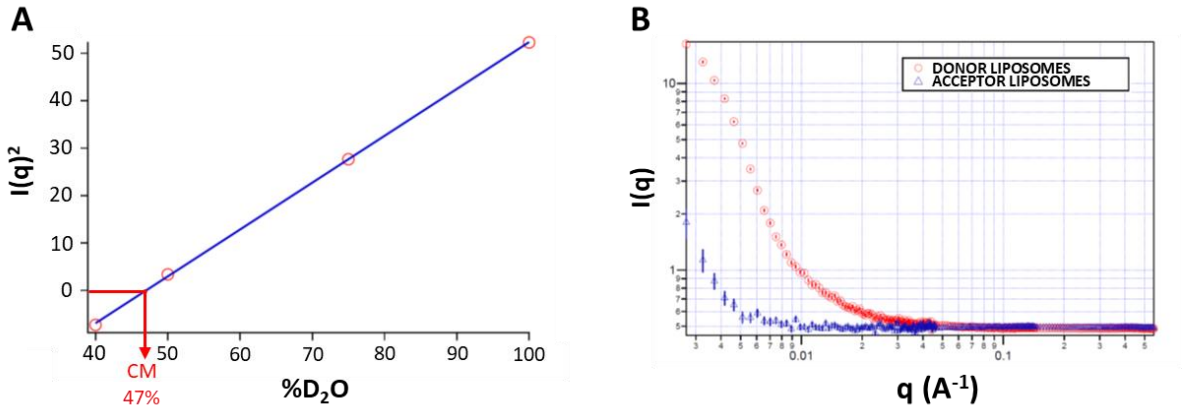

Fig. 1: A. Contrast match (CM) determination of the acceptor liposomes. The CM is achieved with a buffer containing 47% D2O. **B.** Scattering curves of donor (in red) and acceptor (in blue) liposomes in CM buffer.

In order to analyze whether phosphatidylserine (PS) transport mediated by Osh6 can be followed by TR-SANS, we made donor liposomes composed of 30% H-PS, 64% D-phosphatidylcholine (PC) and 6% D-phosphatidylglycerol (PG) and acceptor liposomes composed of 94% D-PC and 6% D-PG. First, acceptor liposomes were diluted at 10mg/mL in buffers with different D_2O contents in order to determine the CM buffer of the acceptor liposomes. A CM buffer containing 47% D_2O was calculated from the experimental curve I(q)1/2=f(%D2O) presented in **Fig. 1A**. The scattering curves of both donor and acceptor liposomes at 10mg/mL in CM buffer was performed. The results presented in **Fig. 1B** confirming that with these conditions, a "high" intensity signal was obtained for donor liposomes compared to the contrast matched acceptor ones as expected. Then, donor and acceptor liposomes at 10mg/mL in the CM buffer were mixed at a 1:1 ratio with the addition of CM buffer (negative control) or Osh6 protein diluted at 0.25µg/µL in CM buffer. The scattering curves of the different samples were

measured at different time points during 12h at 30°C. The normalized intensity (relative to time zero) was stable for both samples during the 12h course and no decrease in intensity was detected (data not shown). These results suggest that 1) in the control experiment, no spontaneous transfer occurs between liposomes, validating our setup and that 2) no lipid transfer mediated by Osh6 was detected. This absence of transfer has been linked 1) to a problem of functionality of the purified protein and/or 2) to the low ratio proteins:lipids (1:25000) used in the protocol. Indeed, in classical in vitro transfer experiments a ratio of 1:400 to 1:1000 is used. Because of technical issues in the purification of Osh6, the yield of purification was very low and we were not able to decrease the proteins:lipids ratio. Because we were not able to further improve our purification yield within the following 2 months, the second session of 24h was cancelled.

Overall, the results from this project were very encouraging for the development of an in vitro lipid transfer assay mediated by proteins by TR-SANS. We were able to validate conditions for which no spontaneous transfer between liposomes was detected and to determine that the proteins:lipids ratio should be also likely be close to 1:1000, as determined for other assays based on fluorescence. As perspectives, other known LTPs, with well established purification protocols in our lab, might be tested as well as a reduction of the liposomes concentration in the reaction mix in order to decrease the ratio proteins:lipids.