Experimental report

17/12/2024

Proposal: 8-04-970 **Council:** 4/2023

Title: Interplay of Mycobacterial Lipids and Host Cell Membranes: StructuralCharacterization using SANS and Spin Echo

Research area: Biology

This proposal is a new proposal

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Samples: mycobacterial lipids
Mycobacterial lipids into the host cell membrane

Instrument	Requested days	Allocated days	From	To
D16	4	4	14/09/2023	18/09/2023
IN15	3	3	18/03/2024 20/03/2024	19/03/2024 22/03/2024

Abstract:

During infection, and during host and drug interactions, mycobacteria (causative agent of TB) respond by remodeling its cell membrane. These remodeling, involves lipid rearrangement with induction of transient non-lamellar and static cubic lipid structures, along with fluctuations in curvature and bending elasticity. This project seeks to explore membrane biophysics of mycobacteria cell wall lipids, which has not yet been explored to the best of our knowledge.

Experiment report 8-04-970 for D16 and IN15

Interplay of Mycobacterial Lipids and Host Cell Membranes: Structural Characterization using SANS and Spin Echo

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Tuberculosis (TB) is the most-deadly malady inflicting millions of people worldwide.[1] Absence of vaccines against TB makes drug therapy the only viable option, however, the emergence of drug-resistance calls to attention development of alternative resistance-free therapies.[2] In this context, membranes and lipids are gaining momentum due to the ability to mitigate emergence of resistance.[3-5]

The main culprit for *Mycobacterial* specie's remarkable defense and a major determinant of virulence is the unusual lipids in its bacterial cell envelope.[6-7] Data from various studies demonstrate that the inner membrane layer consists of phospholipids followed by a peptidoglycan-arabinogalactan layer which is covalently linked to the long chain mycolic fatty acids (C_{60}-C_{90}) constituting the outer membrane.[8-10] Intercalated within outer membrane is a variety of complex lipids including phthioceroldimycocerosate (PDIM), dimycolyltrehalose (TDM), sulfolipids (SL), phosphatidylinositol mannosides (PIMs) and lipoarabinomannan (man-LAM). It has been proposed that the low permeability of the mycobacterial membrane imparts a high degree of intrinsic resistance to most drugs.[11] Interestingly, an important link altered lipid composition and hence different lipid phase structures, membrane properties and drug-resistant phenotype has emerged. For instance, rifampin resistant mycobacterial strain displays cell wall lipid remodeling mediated by altered lipid biosynthesis leading to modulated cell wall permeability to drugs and virulence.[12]

Aim of the Proposal

Work from our lab and others have shown that mycobacterial inner and outer lipid membrane layers in mycobacterium smegmatis (*Msm, lab model of Mtb*) are composed of mutually exclusive lipids, and these dictate the spatially resolved domain organization and associated properties (e.g. order, fluidity and conformation).[13-15] In this work we are proposing to (a) investigate the structural aspects of mycobacterial lipids as encountered under physiological conditions within the bacterial envelope and (b) to query membrane structural modulations induced by the intercalation of mycobacterial lipids into the host cell membrane. The specific objectives are:

1. SANS exploration of the mycobacterial lipid membranes, and correlation with lipidomic profile of the bacteria grown at different conditions (with and without drugs)
2. SANS investigation of the host THP-1 cell membranes upon exposure to exogenous mycobacterial: Insights into membrane-remodeling during infection
3. Spin Echo spectroscopy to investigate bending modulus/rigidity of bacterial and host membranes

Experiments on D16 and IN15

The liposomes of different mycobacterial lipids (OML and IML lipids from bacteria grown in Hypoxic and Normoxic conditions) were freshly prepared in a D_2O-based TRIS $MgCl_2$ buffer at ILL by Aswin

Srivatsav and Dr. Judith Peters. The samples were measured on D16 in the q-range of 0.02 – 1.9 $Å^{-1}$ and at 5 different temperatures (15 °C, 25 °C, 37 °C, 45 °C and 60°C).

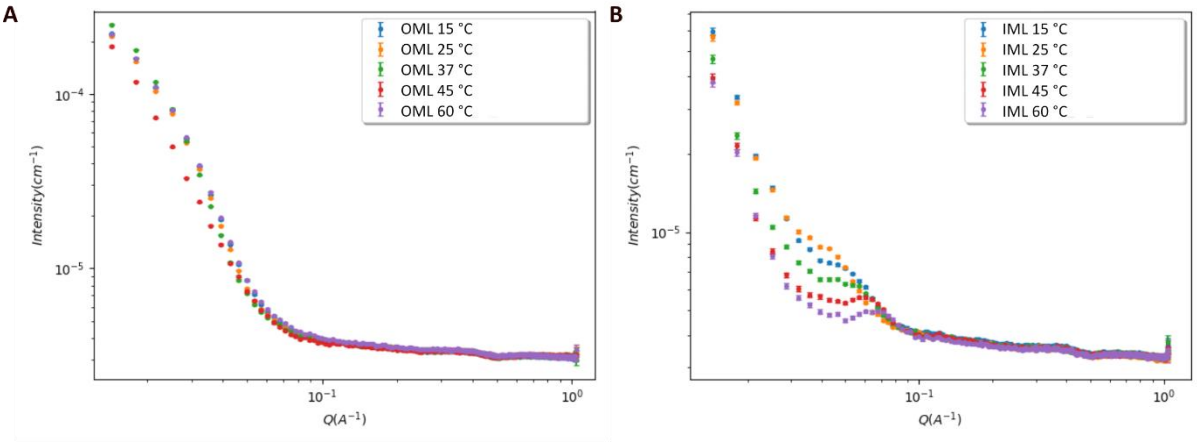

Figure 1: Representative SANS intensity curves of (A) OML and (B) IML lipids in D_2O buffer (50mM Tris and 5mM $MgCl_2$; pD = 7.4) at 5 different temperatures.

In Figure 1, some representative SANS intensity profiles for IML and OML lipids are shown. We can see some small peaks for the IML systems while no prominent peaks are observed for the OML systems. To help us obtain more details from the plots, further analysis to extract more data from the above curves was performed. An example of the Guinier plots created for the OML system is shown in Figure 2.

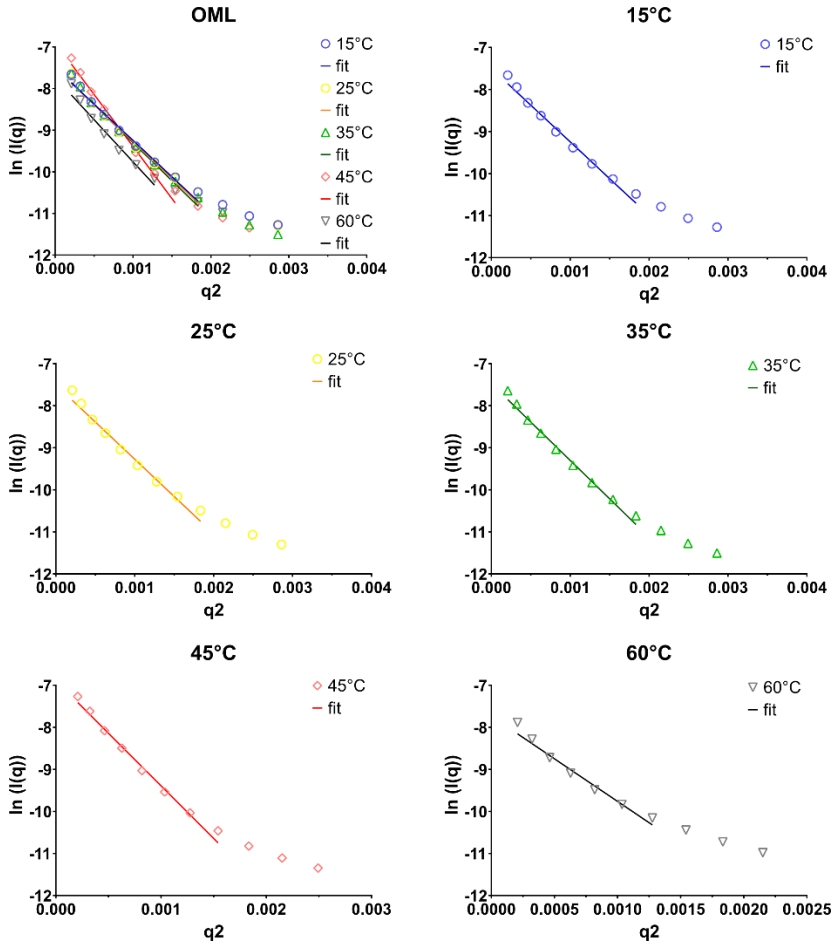

Figure 2: Guinier plots derived from the linear portion of the SANS intensity profiles for the OML system are shown. A similar analysis was done for all other lipid samples.

We were not able to obtain coherent results from the above plots. One of the reasons could be the complexity of the lipids making up these IML and OML systems. This gives rise to a number of variables, such as polydispersity in size, deviation from the spherical shape and more. Due to that, we could not successfully draw any conclusions from the data.

Apart from this, data could not be acquired on IN15 successfully due to an unforeseen reactor shutdown during the acquisition period and decreased quality of the samples a few months later.

References

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