Experimental report

21/04/2017

Proposal: 8-04-765 **Council:** 4/2015

Title: Role of the glycosidic bond of trehalose in the dynamics, rigidity and stability of trehalose mycolates

Research area: Biology

This proposal is a new proposal

Main proposer: Federica MIGLIARDO

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Local contacts: Michael Marek KOZA

Samples: trehalose and glucose mycolates C130H250O15, H2O, D2O

Instrument	Requested days	Allocated days	From	To
IN4	5	0		
IN6	0	5	24/05/2016	30/05/2016

Abstract:

In order to elucidate the role of trehalose in conferring to the mycomembrane of corynebacteria and mycobacteria its extraordinary stability and rigidity and then to go ahead in the understanding of the molecular cofactors responsible for the resistance and virulence of these bacteria, we propose to use the IN4 spectrometer for investigating the dynamical properties of different mixtures of mono- and di-mycolates of glucose and trehalose. More specifically, since trehalose is a disaccharide composed by two glucose units linked by an Ñ-Ñ C1-C1 glycosidic bond, the systems to be investigated are: trehalose monomycolates/trehalose dimycolates at a ratio of 1:1, trehalose monomycolates/glucose monomycolates at a ratio of 1:2. By taking into account that we will collect data on 4 samples at the 5 temperature values of 20-140-260-300-340K (about 4 hours for each temperature), the calibration runs (empty can, transmission and Vanadium), the sample change and temperature stabilization time, we estimate for the required statistics a total of 5 days of beamtime on IN4.

EXPERIMENTAL REPORT

EXPERIMENT N 8-04-765 INSTRUMENT IN6

DATES OF EXPERIMENT 24-30/05/2016

TITLE Role of the glycosidic bond of trehalose in the dynamics, rigidity and stability of trehalose mycolates

EXPERIMENTAL TEAM (names and affiliation)
Federica Migliardo, Dipartimento di Fisica, Università di Messina
Michael Marek Koza, ILL

LOCAL CONTACT Michael Marek Koza

Date of report 21/04/2017

The aim of the performed experiment is to elucidate the role of trehalose in conferring to the outer membrane of mycobacteria its properties of stability and rigidity and hence to better explain the well known extraordinary resistance to mycobacteria and corynebacteria.
The dynamical properties of trehalose monomycolates (TMM)/trehalose dimycolates (TDM) at a ratio of 1:1 and trehalose monomycolates (TMM)/glucose monomycolates (GMM) at a ratio of 1:2 in D_2O and H_2O mixtures (Figure 1) at different temperature values. By such approach we get information about the role of the glycosidic bond in the dynamics, rigidity and stability of trehalose mycolates.

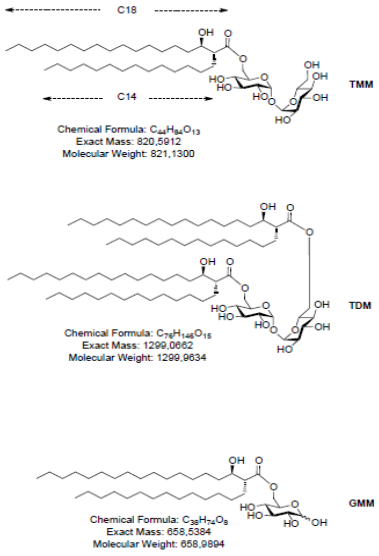

Figure 1 Chemical structure of mycolate mixtures

The presence of a hydrophobic barrier functionally related to the outer membrane of Gram-negative bacteria in mycobacteria and corynebacteria, which is named “mycomembrane”, has been strongly suggested by electrophysiological, structural and biochemical studies, which revealed the presence of very atypical lipids in the form of ester derivatives of mycolic acids.

Mycolates are α-ramified, β-hydroxylated fatty acids with very long chains. In mycobateria, these “free” esters of mycolates include trehalose mono and dimycolates. It has been showed that a strong interaction between the mycolate outer membrane and the underlying polymer is essential for cell envelope integrity.

QENS spectra (Figure 2) have been obtained on TMM-TDM/H_2O, TMM-TDM/D_2O, TMM-GMM/H_2O and TMM-GMM/D_2O in the 200K-340K temperature range. Temperature scans from 2K to 300K have been also performed. Preliminary analyses show that th diffusive dynamics of TMM-GMM is faster than the TMM-TDM one. In addition, the rigidity of this latter mixtures seems higher than the TMM-GMM one. The complete analysis of the data as well as measurements performed by complementary techniques are in progress.

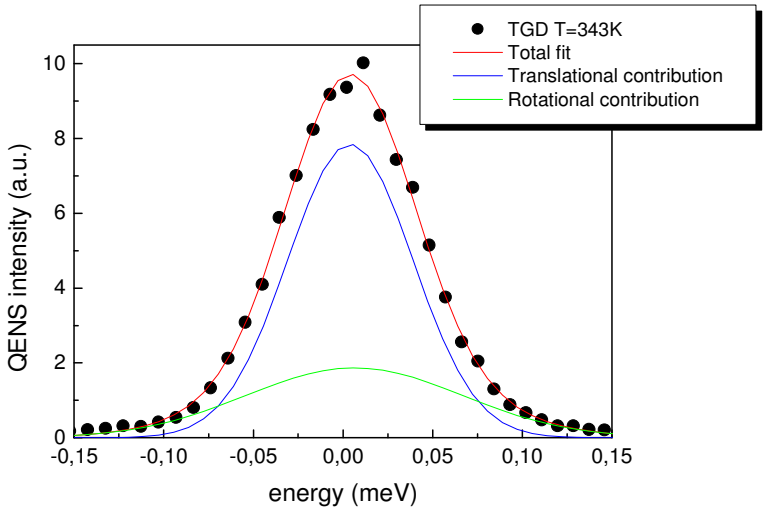

Figure 2 QENS spectrum of TMM-GMM/D_2O mixture at T=343K