Experimental report

13/03/2017

Proposal: 8-03-893 **Council:** 4/2016

Title: Investigation of Phenylketonuria molecular basis : focus on phenylalanine interaction with model membranes

Research area: Biology

This proposal is a new proposal

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Samples: Cholesterol
phenylalanine
phospholipid DMPC
ganglioside GM1

Instrument	Requested days	Allocated days	From	To
D33	3	2	06/10/2016	08/10/2016

Abstract:

Phenylketonuria is one of the most common inherited metabolic disorders (1:10,000 births). A new paradigm for the aetiopathology of phenylketonuria suggests the presence of amyloidlike Phenylalanine assemblies in the brains of transgenic mouse models and patients, possibly shedding light on the selective cognitive deficit associated with this disease. We performed cellular toxicity, X-Ray and microscopy experiments focused on Phenylalanine aggregation mechanism and cytotoxicity. Phenylalanine has been observed to not aggregate in water over long periods, whereas a pronounced propensity to surfaces has been observed, promoting fast aggregation and fibril formation. The extent and effect of interaction of Phe with whole membranes or with membrane lipids is still unexplored. Assessing their mutual structural interaction could provide a clue towards understanding the mechanism of PKU. We plan to investigate by SANS, the interaction of Phe with different model membranes, with biomimetic composition, in bulk solution. The effect of an interfering molecule, as a possible drug, will also be exploited.

Investigation of Phenylketonuria molecular basis : focus on phenylalanine interaction with model membranes

Phenylketonuria is one of the most common inherited metabolic disorders (1:10,000 births). A new paradigm for the aetiopathology of phenylketonuria suggests the presence of amyloid-like Phenylalanine assemblies in the brains of transgenic mouse models and patients, possibly shedding light on the selective cognitive deficit associated with this disease. We performed cellular toxicity, X-Ray and microscopy experiments focused on Phenylalanine aggregation mechanism and cytotoxicity. Phenylalanine has been observed to not aggregate in water over long periods, whereas a pronounced propensity to surfaces has been observed, promoting fast aggregation and fibril formation. The extent and effect of interaction of Phe with whole membranes or with membrane lipids could provide a clue towards understanding the mechanism of PKU. We investigated by SANS the interaction of Phe with different model membranes, with biomimetic composition, in water. We also exploited the effect of doxycycline which was found to prevent Phe cytotoxicity.

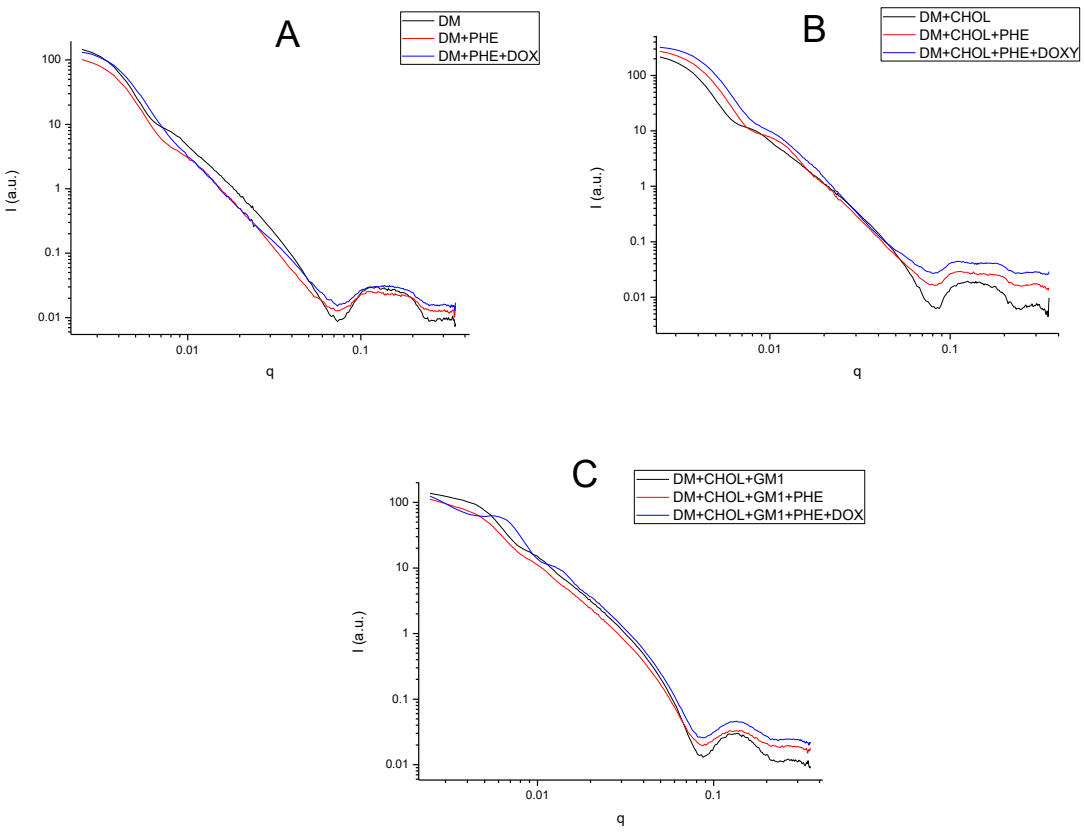

Figure 1. SANS spectra of different model membranes after interaction with Phe (100mM) and Phe:doxy 1:0.005 mol (100mM) solutions in water. The model membranes were composed by A) d_{54}DMPC; B) d_{54}DMPC:cholesterol 10:1.25 mol; C) d_{54}DMPC:Cholesterol:GM1 ganglioside 10:1.25:1 mol. T=25°C.

Figure 1 shows the spectra obtained from the different model systems investigated at 25°C.

Spectra indicate that interaction occurred with all the investigated membranes and that it is different when interaction take place in presence of the very small amount of doxycycline.

All the systems have also been studied at T=50°C, over the lipid chains melting transition, and back to 25°C.

Data fit will shed light on the differences among the various interactions investigated at the different temperatures.