Experimental report

09/02/2018

Proposal: 8-02-775 **Council:** 4/2016

Title: Location of ubiquinone coenzyme Qnin lipid bilayers

Research area: Biology

This proposal is a new proposal

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Samples: POPC
silicon blocks
deuterated d82-POPC
ubiquinone Q10
ubiquinone Q2

Instrument	Requested days	Allocated days	From	To
D17	4	3	12/12/2016	15/12/2016
FIGARO	4	0		

Abstract:

Ubiquinones are linear polyisoprenoid derivatives of quinones that act as electron carriers in many bioenergetics processes, but also as cofactors for enzymes that are targets for a number of anti-proliferative and anti-inflammatory drugs in clinical use. It is generally believed that the branched isoprenyl units of Q10 reside in the hydrophobic centre of membranes, aligned perpendicular to the lipid chains. This raises the question how membrane-bound enzymes such as dihydroorotate dehydrogenase (DHODH), whose catalytic domain resides outside the cell membrane, access the membrane-bound ubiquinones. We are starting a new research project aiming to elucidate the structure and function of membrane-bound DHODH reconstituted into well-defined mitochondrial membrane mimics. As a pre-study we first need to determine the structure of ubiquinone-containing membranes. In this experiment our aim is establish the location of short and long-chain ubiquinones Q2 and Q10 in d82-POPC membranes and deuterated mimics of the inner mitochondrial membrane environment of DHODH.

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D17 - 8-02-775

Abstract:

Ubiquinones are linear polyisoprenoid derivatives of quinones that act as electron carriers in many bioenergetics processes e.g., the respiratory chain, but also as cofactors for enzymes that are targets for a number of anti-proliferative and anti-inflammatory drugs in clinical use e.g., dihydroorotate dehydrogenase (DHODH). It is generally believed that the branched isoprenyl units of Q_{10} reside in the hydrophobic center of membranes, aligned perpendicular to the lipid chains. This raises the question how membrane-bound enzymes such as DHODH, whose catalytic domain resides outside the cell membrane, access the membrane-bound ubiquinones. We are starting a new research project aiming to elucidate the structure and function of membrane-bound DHODH reconstituted into well-defined mitochondrial membrane mimics. As a pre-study, we first needed to determine the structure of ubiquinone-containing membranes. In this experiment, our aim was to establish the location of short and long-chain ubiquinones Q_2 and Q_{10} in d_{82}-POPC membranes and deuterated mimics of the inner mitochondrial membrane environment of DHODH.

Results:

As listed below (Table 1), we measured all 8 samples, in four solvent contrasts each, during the three days allocated on D17. Only the deposition of the deuterated yeast inner mitochondrial membrane (IMM) mimic containing 20 mol% Q_2 was not successful with 70% water found in the hydrophobic chain layer, probably due to lack of sufficient sonication previous to the injection of the vesicles into the reflectivity cell.

Membrane_Q_n (20 mol%)	Contrasts	Completion/Quality
d_{82}-POPC_Q_{10}	D_2O, Cm4, CmSi and H_2O	Yes, Good
d_{82}-POPC_Q_2	D_2O, Cm4, CmSi and H_2O	Yes, Good
hPOPC_Q_{10}	D_2O, Cm4, CmSi and H_2O	Yes, Good
hPOPC_Q_2	D_2O, Cm4, CmSi and H_2O	Yes, Good
d-Yeast IMM mimic_Q_{10}	D_2O, Cm4, CmSi and H_2O	Yes, Good
d-Yeast IMM mimic_Q_2	D_2O, Cm4, CmSi and H_2O	Yes, low coverage of bilayer
h-Yeast IMM mimic_Q_{10}	D_2O, Cm4, CmSi and H_2O	Yes, Good
h-Yeast IMM mimic_Q_2	D_2O, Cm4, CmSi and H_2O	Yes, Good

Table 1. List of samples, solvent contrasts measured and quality of data obtained.

By using the data obtained from the first 4 samples, the synthetic lipid bilayers containing ubiquinones, we were able to determine a different distribution for Q_2 and Q_{10} within the bilayer, even though they were surrounded by the same lipid environment (see figure 1). The data from the short ubiquinone Q_2 could only be fitted with a homogeneous distribution in the lipid chain layer, implying a possible perpendicular orientation to the plane of the membrane, whereas the long ubiquinone Q_{10} was only found in a thin layer in the center of the hydrophobic chain layer, indicating that it lies parallel or nearly parallel to the plane of the membrane at the centre between the two lipid leaflets. The data from the synthetic lipid bilayer with the highest contrast for the location of the ubiquinone Q_{10}, i.e. d_{82}-POPC_Q_{10}, could only be fitted to a model consisting of the following layers (from the substrate towards the solvent): 1) a layer of PC lipid head groups, 2) a layer of 'pure' PO fatty acid chains, 3) a layer where PO and Q_{10} were mixed, 4) a layer of 'pure' PO fatty acids and 5) layer of PC head groups. Such a middle layer feature could not be observed for the POPC bilayers containing Q_2, which was found uniformly distributed within the lipid chain layer.

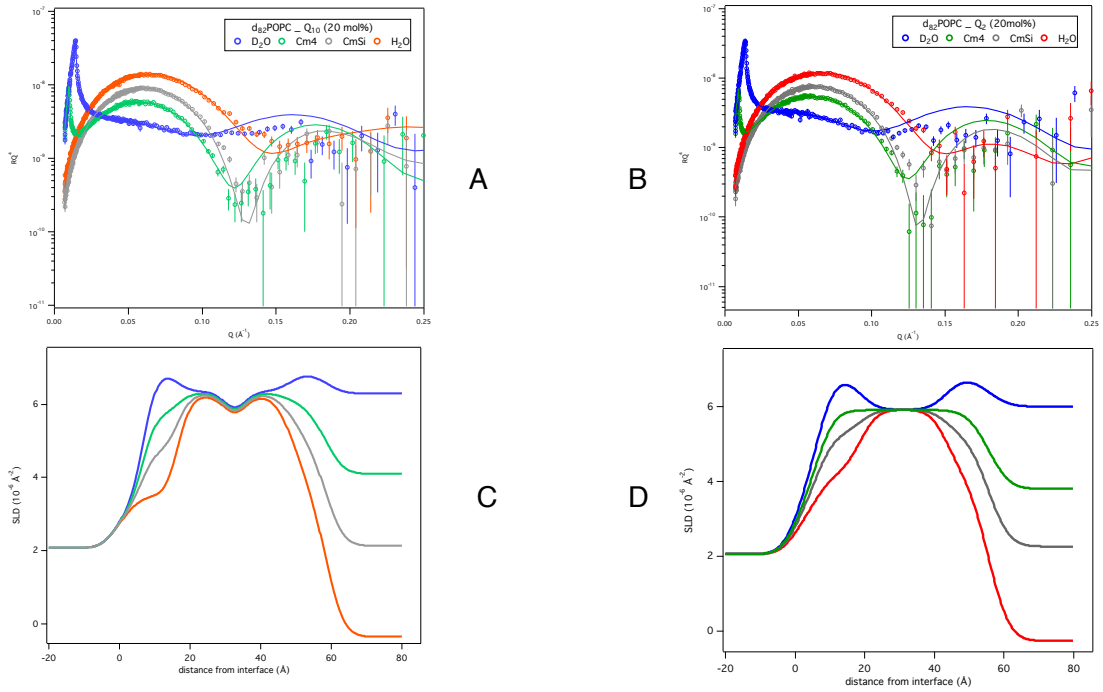

Figure 1. Comparison of the reflectivity data, fits (A & B) and associated scattering length density (SLD) profiles of both d_{82}-POPC_Q_{10} (A & C) and d_{82}-POPC_Q_2 (B & D).

The same observation was made in the bilayers composed of a mixture of yeast phospholipids with a range of different phospholipid head groups and fatty acids (see proposal 8-02-775). It is not possible to conclusively say that the short Q_2 in IMM mimics is homogeneously distributed as the deuterated sample with the best contrast, d-Yeast IMM mimic_Q_2 had only very low surface coverage. The distribution of Q_{10} was broader within the hydrophobic part of the IMM mimics compared to POPC, but there was still a significantly higher amount of Q_{10} found in the bilayer center of (See figure 2). This is consistent with the range of chain lengths chain packing.

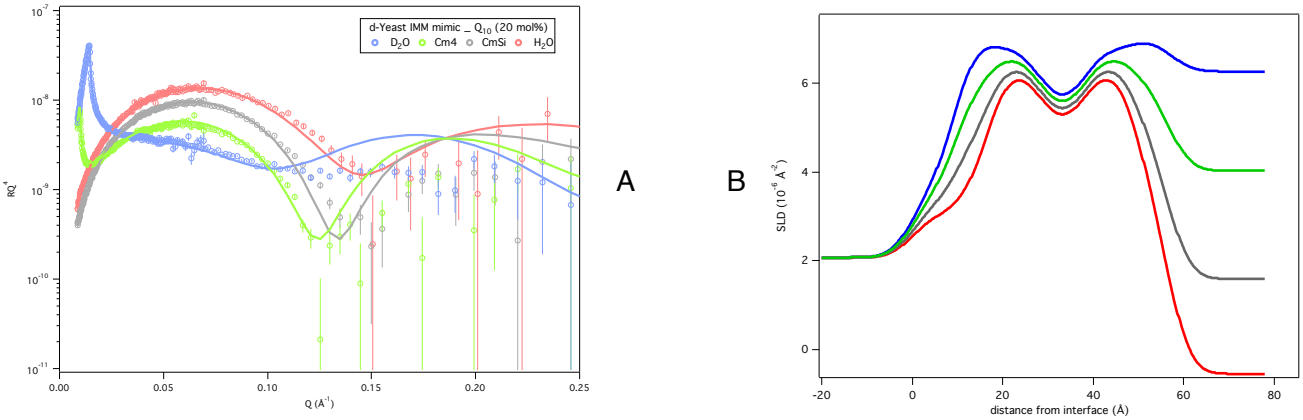

Figure 2. Reflectivity data, fits (A) and associated SLD profile (B) d-Yeast IMM mimic_Q_{10}.

These results are in agreement with the previously proposed location of Q_{10} in the center of the hydrophobic part of synthetic (multi)bilayers, stipulated from neutron diffraction data [1]. However, our results also show the importance of the nature of the lipids found in the bilayers. This will be further investigated as it could be an important feature for the physiological mechanism by which ubiquinone can interact with proteins at the edges of lipid bilayers.

[1] Hauß, T., Dante, S., Haines, T. H., & Dencher, N. A. (2005). Localization of coenzyme Q10 in the center of a deuterated lipid membrane by neutron diffraction. *Biochimica Et Biophysica Acta (BBA) - Bioenergetics*, *1710*(1), 57–62.