Experimental report

12/02/2019

Proposal: 8-02-821 **Council:** 4/2018

Title: Interaction of human dihydroorotate dehydrogenase with ubiquinone in model lipid bilayers

Research area: Biology

This proposal is a new proposal

Main proposer: Juan Manuel OROZCO RODRIGUEZ

Experimental team: Juan Manuel OROZCO RODRIGUEZ
Giovanna FRAGNETO
Robin DELHOM
Hanna WACKLIN-KNECHT

Local contacts: Giovanna FRAGNETO

Samples: silicon wafers
ubiquinone Q10
ubiquinone Q2
h-POPC
h-POPC mimic (Yeast lipid extract)
d9-Ubiquinone Q10
h-DHODH
d82-POPC

Instrument	Requested days	Allocated days	From	To
FIGARO	3	0		
D17	3	3	15/10/2018	18/10/2018

Abstract:

Human dihydroorotate dehydrogenase (DHODH) is an integral membrane protein involved in pyrimidine biosynthesis. DHODH is a well-validated target for anti-inflammatory and anti-proliferative drugs that act as inhibitors. Mutations in DHODH cause Miller syndrome, a rare genetic disorder. DHODH contains two amphipathic alpha helices proposed to be critical for its interaction with the membrane, with its co-substrate ubiquinone Q10 and with inhibitors. Our goal is to investigate the mechanisms by which these interactions occur in a physiologically relevant membrane-bound state consisting of oriented planar lipid bilayers using neutron reflectivity. We have previously performed a successful experiment to investigate the formation and structure of lipid bilayers containing ubiquinone Q10 and a shorter derivative (ubiquinone Q2). As a continuation, we propose to investigate the interaction of N-terminally truncated DHODH (consisting of the catalytic domain and membrane-binding helices) with lipid bilayers containing either ubiquinone Q2 or Q10.

Experimental Report 8-02-821 (D17)

Abstract

Human dihydroorotate dehydrogenase (DHODH) is an integral flavoenzyme of the inner mitochondrial membrane (IMM) that catalyzes the oxidation of dihydroorotate to orotate with the concomitant reduction of coenzyme Q_{10} (ubiquinone Q_{10}) in the *de novo* pyrimidine biosynthesis pathway [1]. DHODH is a well-validated target for anti-inflammatory and anti-proliferative compounds that act as inhibitors of the enzyme for the treatment of autoimmune disorders and certain cancers [2]. In addition, certain mutations in the gene encoding human DHODH have been identified as the cause of Miller syndrome, a rare Mendelian disorder characterized by head and limb abnormalities [3]. Our goal is to use neutron reflectometry in order to investigate the mechanisms by which DHODH interacts with ubiquinone, with IMM lipids and with inhibitors, in a non-crystalline, physiologically relevant membrane-bound state.

Results

Due to an unexpected reactor shutdown, our beamtime was drastically reduced, from 3 days to only 1 and this one with only half the normal power. Consequently, we were only able to measure 2 out of 5 samples (Table 1), h-POPC, and a mitochondrial mimic, h-POPC_Q_{10} (80 mol% POPC, 10 mol% cardiolipin (CL) and 10 mol% Q_{10}.) Four contrasts were measured before and after protein addition for every bilayer in 10 mM Tris-HCl, 100 mM NaCl, 2 mM $CaCl_2$, pH 7.4.

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

Membranes_Q_n (10 mol%)	Contrasts before interaction	Protein	Contrasts after interaction	Completion/Quality
d_{82}-POPC_Q_2	H_2O, CmSi, Cm4, D_2O	+ DHODH	H_2O, D_2O	Not measured
d_{82}-POPC_Q_{10}	H_2O, CmSi, Cm4, D_2O	+ DHODH	H_2O, D_2O	Not measured
h-POPC_Q_2	H_2O, CmSi, Cm4, D_2O	+ DHODH	H_2O, D_2O	Not measured
h-POPC_Q_{10}	H_2O, CmSi, Cm4, D_2O	+ DHODH	H_2O	Measured / No interaction
h-POPC mimic_Q_{10}	H_2O, CmSi, Cm4, D_2O	+ DHODH	H_2O, D_2O	Measured / No interaction

Figure 1 displays the reflectivity curves of the bilayers formed from vesicles consisting of either h-POPC (Fig. 2A) or the h-POPC_Q_{10} mixture (Fig. 2B), before and after addition of 0.4 mg/mL DHODH. As can be observed, addition of the enzyme did not result in changes in the reflectivity profiles of the bilayers, indicating that DHODH was unable to bind and interact with the membranes.

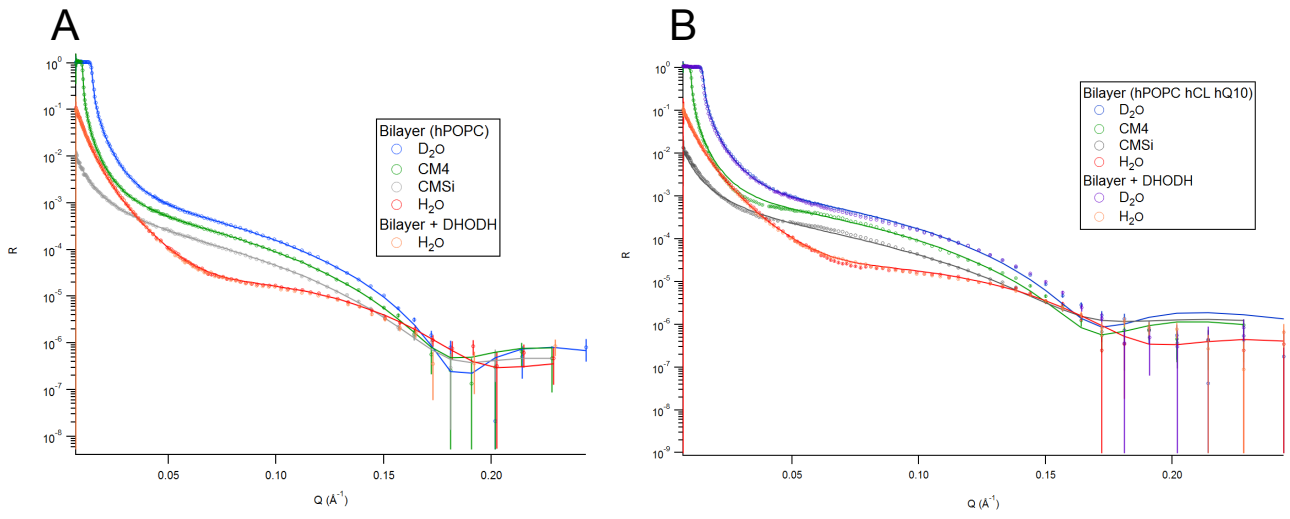

Figure 1. Reflectivity curves of bilayers formed from h-POPC vesicles (A) or h-POPC mimics (B), before and after interaction with DHODH.

We had previously observed a reversible interaction of DHODH with these bilayers using QCM-D, so this was somewhat surprising. We have since then identified some of the reasons why the protein failed to bind the bilayers during the NR measurements. The following factors are very likely to be responsible:
1) *Differences in the ratio of protein mass in solution to surface area*. The silicon blocks used for NR measurements have a surface area of 40 cm^2 (80 × 50 mm), while the quartz crystals used for QCM-D measurements are 1 cm^2 in area. Previous QCM-D experiments indicated a strong binding between bilayers consisting of 80% POPC, 10% CL and 10% Q_{10} and DHODH diluted to a final concentration of 0.1 mg/mL. In a typical QCM-D experiment, the total mass of protein added per unit area is up to 200 µg/cm^2. In contrast, the maximum amount of protein added per unit area was 10 µg/cm^2, which is 20-fold lower.

2) *The presence of calcium in the buffer*. The buffer used throughout the NR measurements contains 2 mM $CaCl_2$. In QCM-D trials performed afterwards (Fig. 2), we were able to observe that even low concentrations of calcium chloride (5 mM) decrease the affinity between the bilayers and DHODH when compared with enzyme added in the absence of calcium. Furthermore, bound DHODH can be removed by rinsing with buffer containing 5 mM $CaCl_2$.

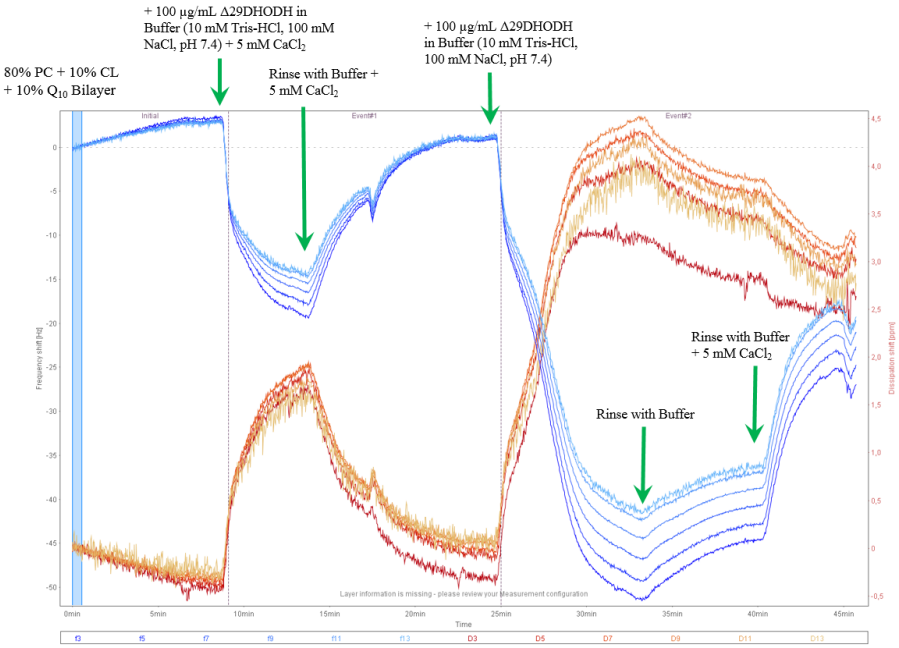

Figure 2. QCM-D analysis of the effect of calcium chloride on the binding between DHODH and lipid bilayers formed from vesicles consisting of 80% POPC, 10% CL and 10% Q_{10}.

For future NR measurements, calcium will be omitted from the buffers used for rinsing and for diluting the protein. A higher mass of protein per unit area will be added (at least 50 µg/cm^2). We are confident that we can come to conclusive results on binding of DHODH, following this strategy, when we get replacement for our lost beamtime and next applied beamtime. In this respect the experiment has delivered valuable information for us, despite the loss of most of the experimental time.

References

1. Loffler, M., et al., Trends Mol Med, 2005. 11(9): p. 430-7.
2. Sykes, D.B., et al., Cell, 2016. 167(1): p. 171-186.e15.
3. Ng, S.B., et al., Nat Genet, 2010. 42(1): p. 30-5.