Experimental report

28/05/2020

Proposal: 8-02-858 **Council:** 10/2018

Title: Interaction of human dihydroorotate dehydrogenase with ubiquinone anddihydroorotate in mimics of the inner mitochondrial membrane

Research area: Biology

This proposal is a continuation of 8-02-775

Main proposer: Wolfgang KNECHT

Experimental team: Juan Manuel OROZCO RODRIGUEZ
Oliver BOGOJEVIC
Hanna WACKLIN KNECHT
Wolfgang KNECHT

Local contacts: Giovanna FRAGNETO

Samples: Silicon Blocks
Dihydroorotate dehydrogenase (hydrogenous)
Phospholipid mixture (hydrogenous)
Ubiquinone d9Q10 (head-deuterated)
Ubiquinone Q10 (hydrogenous)
Dihydroorotic acid (hydrogenous)
Phospholipid mixture (deuterated)

Instrument	Requested days	Allocated days	From	To
FIGARO	3	0		
D17	3	3	01/07/2019	04/07/2019

Abstract:

Human dihydroorotate dehydrogenase (DHODH) is an integral membrane protein found in the inner mitochondrial membrane (IMM). DHODH is a flavoenzyme that catalyzes the oxidation of dihydroorotic acid (DHO) with the simultaneous reduction of ubiquinone (coenzyme Q10). DHODH is a well-validated drug target for the treatment of autoimmune disorders. In addition, mutations in the DHODH gene are the cause of Miller Syndrome, a Mendelian disorder characterized by abnormalities of the head and limbs. DHODH consists of a mitochondrial signal (MS), a transmembrane helix domain (TM), two amphipathic alpha helices and a catalytic domain. We propose to continue our investigation of the interaction between the soluble form of DHODH (lacking the MS and TM segments) and supported lipid bilayers consisting of natural phospholipid mixtures derived from yeast (instead of synthetic lipids) mimicking the composition of the IMM. The bilayers will incorporate Q10 (either hydrogenous or partially deuterated). Finally, the soluble substrate (DHO) will be added to determine whether substrate addition results in changes in the location and conformation of the enzyme and Q10.

Experimental Report 8-02-858 (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

For this experiment, we investigated the interaction between truncated human DHODH (Δ29DHODH) lacking the N-terminal mitochondrial signal peptide and transmembrane domain and hydrogenous polar lipid mixtures extracted from the membranes of the yeast *Candida glabrata* (designated $h_3$pol), both in the absence and presence of ubiquinone Q_{10} (10 mol%).

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, as well as after rinsing.

The table below summarizes the measurements performed.

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

Lipids	Contrasts before protein addition	Protein	Contrasts after protein addition	Contrasts after rinsing
h3pol	H_2O, CmSi, Cm4, D_2O	Δ29DHODH	H_2O, CmSi, Cm4, D_2O	H_2O, CmSi, Cm4, D_2O
h3pol_Q_{10}	H_2O, CmSi, Cm4, D_2O	Δ29DHODH	H_2O, CmSi, Cm4, D_2O	H_2O, CmSi, Cm4, D_2O

Figure 1 displays the reflectivity curves of the bilayers formed from vesicles consisting of the h3pol lipid mixture before and after addition of 0.4 mg/mL Δ29DHODH. As can be observed, addition of the enzyme results in significant changes in the reflectivity profiles of the bilayers (particularly visible in the D_2O and CM4 contrasts), indicating that DHODH does bind and interact with the membranes, and remains bound even after rinsing. Preliminary fitting suggests that the enzyme binds as two layers with a thickness of 45 Å and a high water content (>90%).

Figure 2 displays the reflectivity curves of the bilayers formed from vesicles consisting of the h3pol_Q_{10} mixture before and after addition of 0.4 mg/mL Δ29DHODH. As can be observed, addition of the enzyme results in significant changes in the reflectivity profiles of the bilayers, indicating that DHODH does bind and interact with the membranes, and remains bound even after rinsing. Preliminary fitting suggests that the enzyme binds as three layers with a thickness of 40 Å with a progressively higher water content (as low as 85% in the inner layer and as high as 99% in the outer layer).

Data refinement is ongoing.

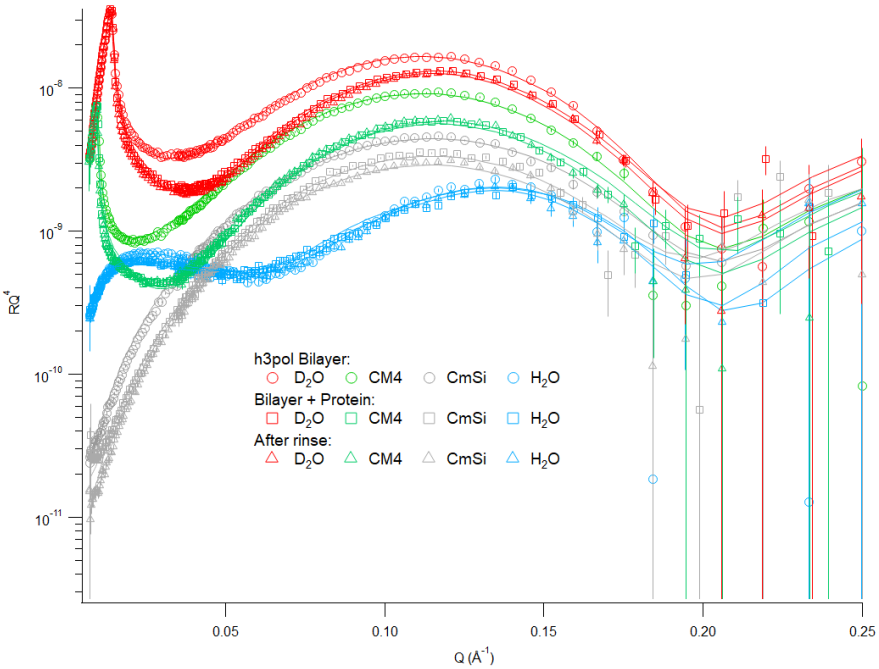

Fig 1. Reflectivity curves and preliminary fits corresponding to the interaction between bilayers consisting of polar lipids extracted from *C. glabrata* and truncated human DHODH.

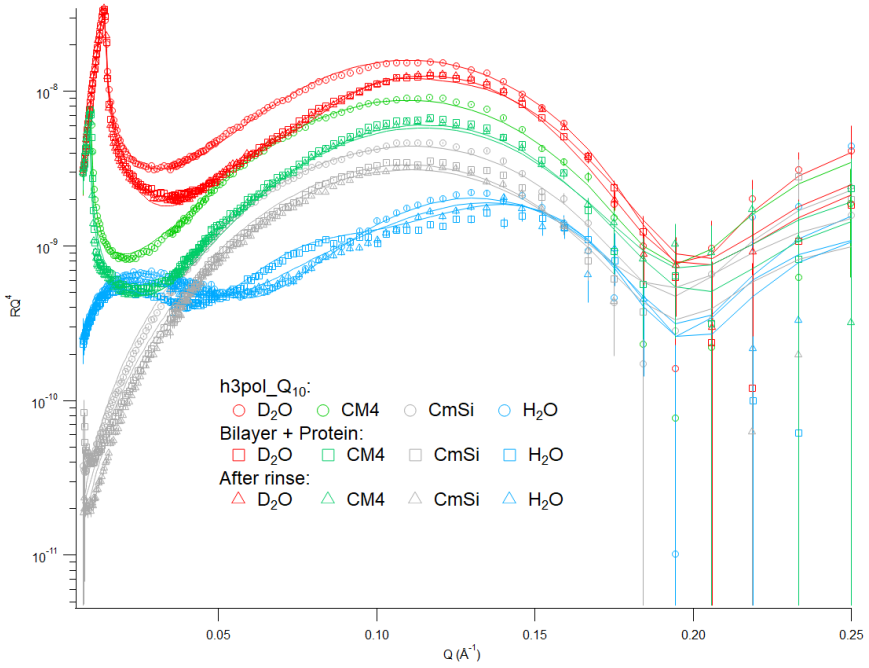

Fig 2. Reflectivity curves and preliminary fits corresponding to the interaction between bilayers consisting of polar lipids extracted from *C. glabrata* supplemented with 10 mol% ubiquinone Q_{10} and truncated human DHODH.

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.