ESRF	Experiment title: BAG: Frankfurt Cytochrome bc_1-complex of *Saccharomyces cerevisiae* (Fv-fragment mediated crystallization)	Experiment number: LS-1514
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Report: The mitochondrial cytochrome bc_1-complex, an oligomeric membrane protein, is one of the fundamental components of the respiratory chain. It catalyzes electron transfer from ubiquinol to cytochrome *c*, while the process is coupled to electrogenic translocation of protons across the inner mitochondrial membrane. The proton motive Q-cycle is a widely accepted model for the functioning of this protein. Aiming at a detailed understanding of its mechanism we study the cytochrome bc_1-complex from the yeast *S. cerevisiae*.

The cytochrome bc_1-complex from *S. cerevisiae* was crystallized with the help of a specifically binding antibody-Fv-fragment. The crystals belong to the space group C2 and diffract x-rays better than 2.2 Å resolution at synchrotron radiation sources. We recently determined the strucure of the complex using multiple isomorphous replacement. The structure was refined to a crystallographic R-factor of 21.1% (R_{free} 25.4 %) [1]. One monomer of the homodimeric complex consist of nine polypeptides of the enzyme plus two chains of the Fv-fragment. 2229 amino acid residues, 4 redox-cofactors, the natural substrate coenzyme Q6 and the inhibitor stigmatellin as well as 371 water molecules and five phospholipid molecules are present per monomer.

Since there are no cryo-methods available for these crystals, measurements still have to be performed at 4°C and the quality of diffraction rapidly decreases upon exposure to radiation. This can be compensated by translation of crystals after few exposures and by scaling data of several crystals. After a period of testing for suitable measurement conditions at the ID14EH2 (1/16 bunch mode) attenuation had to be introduced to allow reasonable data collection.

The structure of the yeast cytochrome bc_1 complex contains five well defined and tightly bound phospholipid molecules. One of the phospholipid molecules was identified as cardiolipin. Their specific binding sites suggest specific roles in function and assembly of the complex. A new protein preparation procedure was established to improve retainment of phospholipids during the protein purification. A native data set of these crystals was collected [2.5 Å resolution, 7.9 % R-merge (overall), 89 % completeness (overall), 1.3 <I/sigI> (outer shell)].

A data set of crystals at lowered pH was measured, to test the effect of pH on the substrate binding pockets and on buried water chains, which are proposed to be of importance for proton exchange [1,2]. [2.8 Å resolution, 8.4 % R-merge (overall), 86 % completeness (overall), 1.3 <I/sigI> (outer shell)].

Crystals were soaked with a cardiolipin specific reagent to identify additional binding sites of this phospholipid [2.8 Å resolution, 8.2 % R-merge (overall), 84 % completeness (overall), 1.1 <I/sigI> (outer shell)].

Fv-fragment mediated crystallization allows the reproducible production of well diffracting crystals of the yeast cytochrome bc_1 complex. A combined approach of X-ray crystallography, biochemical analysis, site-directed mutagenesis and spectroscopy is used to study mechanism and structure/function relationship of this highly important membrane protein. In further experiments we will focus on high resolution data sets with and without Qo-site inhibitors to elucidate the process of quinol oxidation and to collect data at different redox states. Crystallization of protein from cytochrome bc_1 complex mutants is in preparation.

Furthermore, different type of crystals of the NhaA Na^+/H^+-antiporter from *Escherichia coli* were tested during this beamtime. However, quality of these crystals is still unsufficient.

[1] C. Hunte, T., J. Koepke, C. Lange, T. Rossmanith and H. Michel. Structure at 2.3 Å resolution of the cytochrome bc_1 complex from the yeast *Saccharomyces cerevisiae* co-crystallized with an antibody Fv-fragment. Structure (accepted for publication 2000)
[2] C. Hunte, C. Lange, J. Koepke, H. Michel. X-ray structure of the bc_1 complex suggests roles for phospholipids. Science, submitted