ESRF	**Experiment title:** BAG: Frankfurt Cytochrome bc_1-complex of *Saccharomyces cerevisiae* (Fv-fragment mediated crystallization)	**Experiment number:** LS-1661
Beamline: ID14/EH3	**Date of experiment:** from: 8.7.2000 to: 9.7.2000	**Date of report:** 25.8.00
Shifts: 3	**Local contact(s):** S. Arzt	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

C. Hunte *, C. Lange*, H. Michel

Max-Planck-Institute of Biophysics
Heinrich-Hoffmann-Str.7 60528 Frankfurt, Germany
e-mail: Carola.Hunte@mpibp-frankfurt.mpg.de

Report:

We recently solved the structure of the cytochrome bc_1-complex from *S. cerevisiae* bound to an antibody Fv fragment [1,2]. This mitochondrial 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 exact molecular interaction of the enzyme with the substrate cytochrome *c* is not known. We obtained small crystals of a ternary complex consisting of cytochrome bc_1-complex, Fv fragment and cytochrome *c*. The crystallization attempts resulted previously in two type of crystals: I (space group C2) and II (space group P21). Crystallization conditions were difficult to control and result mostly in a mixture of both space group. Furthermore, no electron density could be found for cytochrome c when trying to solve the structure by molcular replacement. We now improved crystallization conditions favouring stronger interaction of cytochrome c with the cytochrome bc_1-complex. These type III crystals can be grown reproducably. They are small (approximately 0.2 x 0.2 x 0.2 mm), but they were reasonably stable in a few pre-tested cryo protecting agents. Therefore, we tried in the beginning to collect data at cryo-temperature. This approach was not successful. Data collection was then performed at 4 °C, the standard condition for measuring data of crystals

of the yeast cytochrome bc_1-complex. As the cooling device was not available (it was in use at BM14) there was a delay until a replacement was available from another beamline. One data set was collected, merged and scaled from ten small crystals. The crystals belong to the space group P21 with unit cell parameters a=147Å, b=166Å, c=196Å, β=104°. Although the crystals diffract better than 3 Å resolution, due to their small size and fast loss of diffraction quality the resolution of the data set was limited. The native data includes data between 30 and 3.65 Å resolution. It has 12.1 % R-merge (overall), 95.2 % completeness and 3.3 I/sigI> (outer shell). Currently, structure solution is attempted by molecular replacement. The crystal lattice has a dimer of the complex in the assymetric unit. Furthermore, the crystals have a low solvent content (~65%). It cannot be concluded yet, if cytochrome *c* is bound in an ordered manner.

The only known structure of a co-complex from an enzyme and cytochome *c* is that of cytochrome c peroxidase and cytochrome c. No structures are available yet of co-complexes between components of the respiratory chain and cytochrome c.

We are now improving the very reproducable crystallization conditions to obtain either larger crystals or to allow cryo-cooling of the crystals so that collection of a high resolution data is possible.

[1] C. Hunte, T., J. Koepke, C. Lange, T. Rossmanith and H. Michel (2000) 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 8, 669-684.

[2] J. Nett, C. Hunte, B.L. Trumpower, Changes to the length of the flexible linker region of the Rieske protein impair the interaction of ubiquinol with the cytochrome bc1 complex Eur. J. Biochem. (in press)