



Experiment title: Cytochrome <i>bc</i> ₁ -complex from <i>S. cerevisiae</i> NhaA from <i>E. coli</i>	BAG: Frankfurt	Experiment number: LS-1930
Beamline: ID14/EH3	Date of experiment: from: 20.4.2001 to: 21.4.2001	Date of report: 28.8.01
Shifts: 3	Local contact(s): H. Belrhali	<i>Received at ESRF:</i>

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Report:

We recently solved the structure of the cytochrome *bc*₁-complex (QCR) from *S. cerevisiae* bound to an antibody Fv fragment [1]. This mitochondrial multisubunit 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* was not known up to now. We obtained small crystals of a ternary complex consisting of QCR, Fv fragment and cytochrome *c*. Only two crystals were large enough for data collection at 4°C, cryo conditions are not available. During the last beamtime (report 26.02.01) data collection could be only completed for the first crystal due to a major power cut at ESRF. The second crystal was stored and data collected during the reported beamtime (30-2.97 Å, 83 % completeness, 13.3 Rsym). However, these data could not be scaled to the first data set and did not improve the results. Using the first data set the structure could be solved by molecular replacement using the high resolution structure of yeast QCR [1,2]. The interaction critical for electron transfer is stabilized mainly by non-polar forces. The close spatial arrangements of the cofactors unexpectedly suggests a direct heme-to-heme electron transfer at a high transfer rate. Evidence is given that QCR might be competent to control

cytochrome c reduction. In addition, tightly bound phospholipid molecules were identified in the original high resolution structure of yeast QCR [1,3]. Their binding sites and conformations are unexpected and remarkable, and provide hints on their function in the mechanism, assembly, and stability of the complex.

Nha is the main Na^+/H^+ antiporter in *Escherichia coli*. It is the first antiporter that yields crystals, both on its own and in complex with Fv or Fab fragments. The diffraction quality of several small crystals resulting from different crystallization conditions were tested. The crystal packing has been improved, higher diffraction limits up to 4.4 Å resolution have been obtained. However, these crystals are anisotropic and have a lower diffractions limit of 12 Å resolution. Further improvements of the crystals is required for structure solution of the antiporter.

Translocation of nuclear-encoded preproteins across the outer membrane of mitochondria is mediated by the multisubunit transmembrane TOM complex. 3D crystals of a core complex have been obtained (S. Schmitt, S. Nussberger, W. Neupert, Munich). The diffraction quality of several crystals have been tested. The crystals are ordered but show poor resolution. Crystallization conditions have to be improved.

[1] C. Hunte, T., J. Koepke, C. Lange, T. Rossmann 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] Lange, C. and Hunte, C. Rapid reduction of cytochrome c by cytochrome bc_1 complex and its half-of-the sites binding; submitted

[3] Lange, C., Nett, J.H, Trumpower, B.L. and Hunte, C. Specific roles of protein-phospholipid interactions in the yeast cytochrome bc_1 complex structure; submitted