



Experiment title:
Cytochrome bc_1 -complex (F_v-mediated crystallization)

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LS-991

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BM14

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Local contact(s): Andrew Thompson

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Names and affiliations of applicants (* indicates experimentalists):

Prof. Dr. Hartmut Michel
Dr. Carola Hunte *
Dr. Juergen Koepke *
Dr. C. Roy D. Lancaster *

Report:

The mitochondrial cytochrome bc_1 -complex, an oligomeric membrane protein complex, 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 the cytochrome bc_1 -complex. A detailed structure of this protein complex is not only of interest to understand the mechanism of the enzyme, but will aid the development of fungicides, as this membrane protein is the target of a new class of fungicides, the strobilurines.

Crystals of the cytochrome bc_1 -complex of *Saccharomyces cerevisiae* were obtained by co-crystallization of the membrane protein complex with the help of a specifically binding antibody-Fv-fragment. The crystals were grown in the presence of stigmatellin, an inhibitor of the membrane protein complex.

X-ray measurements were performed at ESRF BM14 using cryostat cooling adjusted to 4 °C. Diffraction of the crystals was noted up to 2.5 Å, thereby confirming data obtained at home radiation sources. However, stability of the crystals exposed to the x-ray beam was low. Therefore, using carbon film attenuation of the x-ray beam and a CCD-detector a complete data set was collected only up to 3 Å resolution. The crystals belong to the space group C2. Cell dimensions of $a=214$, $b=160$, $c=149$, $\alpha=90$, $\beta=117$, $\gamma=90$ were determined.

Structure determination was tried by molecular replacement using the data set obtained and the fragmentary coordinates of the bovine cytochrome bc_1 -complex provided by Xia and in addition with chicken cytochrome bc_1 -complex coordinates supplied by Berry. Using the program systems X-PLOR and AMORE for molecular replacement identical solutions were found for the rotation and translation searches. However, probably caused by structural differences, no new structural feature appeared. Therefore, higher resolution data sets and independent phase information from heavy atom derivatives are needed to solve the structure. Progress is hampered by the facts that the crystals cannot be frozen and for high resolution data collection only one or two exposures are possible per crystal position.

Apart from heavy atom derivative data sets, more than ten data sets (plus/minus inhibitors, plus/minus substrates, different redox conditions) will have to be collected.

The yeast protein is biochemically, spectroscopically and genetically the best studied cytochrome bc_1 -complex. It is therefore necessary to collect as many data as possible from this protein complex in order to make full use of the available data and to understand the mechanism of this important membrane protein.