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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*.

We recently solved the structure of the cytochrome bc_1-complex from *S. cerevisiae* bound to an antibody Fv fragment [1]. This structure showed for the first time binding of the natural substrate coenzyme Q6 at the Qi site and suggested possible proton uptake pathways. However, the orientation of the ubiquinone headgroup within the ringplane is still not unequivocally defined. For a further description of the Qi-site and analysis of site specific inhibitors we collected two data sets [a1s: 2.5 Å resolution, 6.8 % R-merge (overall), 93.8 % completeness (overall), 1.7 <I/sigI> (outer shell); fem: 2.95 Å resolution, 6.0 % R-merge (overall), 76.4 % completeness (overall), 1.3 <I/sigI> (outer shell)].

The structure of the yeast cytochrome bc_1 complex contains five well defined and tightly bound phospholipid molecules Their specific binding sites suggest specific roles in function and assembly of the complex (Hunte et al., in preparation). A new protein preparation procedure was previously established and had been shown to improve

retainment of phospholipids in the crystals (LS-1514). We tried to selectively displace one of the phospholipid molecules and collected a native data set of crystals of this protein [2.5 Å resolution, 6.2 % R-merge (overall), 92 % completeness (overall), 1.14 <I/sigI> (outer shell)].

Furthermore, we obtained crystals of a ternary complex consisting of cytochrome bc_1-complex, Fv fragment and cytochrome *c*. The crystallization attempts resulted 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 groups. We collected a data set of type I crystals [2.8 Å resolution, 6.2 % R-merge (overall), 97.8 % completeness (overall), 1.5 <I/sigI> (outer shell)]. The unit cell parameters a=216Å, b=164Å, c=149Å, β=118° are slightly larger than those of the original co-complex crystals. Although the crystals contain cytochrome *c* (as shown by biochemical analysis), no electron density could be found for cytochrome *c* when trying to solve the structure by molcular replacement. We are currently improving crystallization conditions to allow tighter binding of cytochrome *c* to the complex.

All data collection was performed at 4°C.

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. 1. The molecular process of quinol oxidation including the movement of the extrinsic domain the subunit RIP1 [2] is still not fully understood. Therefore, we will focus our work in the near future on analysis of the Qo site. We already obtained crystals with an inhibitor analoguous to quinol. 2. Furthermore, we want to elucidate the specific function of tightly bound phospholipid molecules by analyzing the structure of deletion mutants (crystallization in progress). 3. We are planning to analyze the structure of mutants, which effect the proton uptake and release pathways, which were suggested by the high resolution structure [1].

[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 bc_1 complex. Eur. J. Biochem. (in press)