ESRF	**Experiment title:** Structure Determination of Mitochondrial Cytochrome b/cl Complex from Bovine Heart Using X-ray Diffraction.	**Experiment number:** LS 403
Beamline: ID2, BL4	**Date of Experiment:** from: April 7th, 1996 to: April 9th, 1996	**Date of Report:** February 17th,1997
Shifts: Six	**Local contact(s):** Jeffery Shaw	*Received at ESRF :* 2 7 FEV. 1997

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Report: Cytochrome b/cl complexes (ubiquinol:cytochrome c oxidoredutases, b/cl) are essential components of the respiratory chains of all organisms, and of the photosynthetic apparatus of purple bacteria; green plants also use the closely related cytochrome b_6/f complex for energy conversion. These complexes are integral membrane proteins; they transfer electrons from ubiquinol to cytochrome c and simultaneously pump protons across the membrane. The b/cl from bovine heart mitochondria consists of eleven protein subunits with a total molecular weight close to 250 kDa. The structure solution of this membrane complex is expected to have strong impact on related research area such as bioenergetics and also, through the subunits core1 and core2, on mitochondrial protein import. The size and composition of the b/cl pose significant crystallographic challenges. Crystals of this complex were reported by four groups (1) over the last nine years. Recent successes in growing quality b/cl crystals and in stabilizing these crystals in the X-ray beam as well as in searching for heavy atom derivatives in our laboratories have enabled us to find an initial phase solution to the structure determination of the b/cl (2).

The b/cl crystals grown in our laboratories are 0.5 to 0.7 mm in size, and have the symmetry of space group I4,22 and large unit cell dimensions of $a = b = 154$ A and c = 600 A. As a result, the diffraction of these crystals is intrinsically very weak, making it impractical to attempt data collection using a laboratory X-ray source. Therefore all diffraction experiments with the b/cl crystals have been performed at beam lines of various synchrotrons in the US and Europe. The best diffraction of b/cl crystals at the

beam lines X12B and X4A of National Synchrotron Light Source (NSLS) at Brookhaven National laboratory (BNL), and 7-A of Stanford Synchrotron Radiation Laboratory (SSRL) is around 3.3 A resolution for both b/cl native and derivatized crystals. As a result, it was very difficult to interpret the initial MIR electron density maps. In April of 1997, we were awarded six shifts (starting April 7th, 1996, ending April 9th, 1996) of beam time at beam line ID2, BL4 of European Synchrotron Radiation Facility (ESRF) to carry out diffraction experiments on the b/cl crystals. X-ray diffraction spots as far out to as 2.56 A, resolution were observed for some b/cl crystals at this beam line with one minute of exposure time at wavelength of 0.99 A, where the highest beam brilliance can be obtained. Although high resolution diffraction of b/cl crystals lasted only a few frames at this beam line even under cryo conditions around l00°K, most crystals were able to give diffraction better than 3.3 A, for about 30 minutes. Approximately 30 crystals were tested during the experiments; ten data sets were actually collected to different degrees of completeness; among them only four data sets can be merged with the existing native data set with reasonably low merging R-factors. The statistics of the combined data set are given in the table at the end of this report. The native data set has low overall completeness, especially at high resolution shells, nevertheless, it provided valuable high resolution information allowing phase extension to 2.8 A, resolution and enabling successful amino acid sequence assignments to density modified MIR maps. Currently, over 1500 residues have been assigned to the electron density out of 2200 amino acid residues present in this complex, another 300 residues have been traced but not assigned in sequence. There are about 300 amino acid residues still missing. A paper describing the overall structure of the b/cl complex has been submitted for publication.

Table Statistics of Diffraction Data from Native b/cl Complex Crystals*

Resolution limits (A)	Completeness (%)	R-factors (linear)	Resolution limits (A)	Completeness (%)	R-factors (linear)
60.0 - 6.4 1	99.5	0.117	3.21 - 3.08	76.1	0.425
6.41 - 5.09	99.9	0.116	3.08 - 2.98	67.2	0.441
5.09 - 4.45	99.9	0.130	2.98 - 2.88	61.2	0.526
4.45 - 4.04	99.9	0.172	2.88 - 2.80	52.2	0.623
4.04 - 3.75	100.0	0.273	2.80 - 2.73	46.4	0.803
3.75 - 3.53	100.0	0.382	2.73 - 2.66	40.6	0.804
3.53 - 3.35	100.0	0.583	2.66 - 2.60	32.2	0.857
3.35 - 3.21	88.8	0.722	Overall	77.9	0.176

* σ = -3 cutoff was used in calculating R factors.

References

1. ***Yu.*** & ***Yu*** (1993) ***J. Bioenerg. Biomember.*** 25, 259-274. Kubota et al. (1991) ***J. Mol. Biol.*** 221, 379-382. Berry et al (1992) ***J.*** Mol. ***Biol.*** 224, 1161-1166. Yu et al (1994) ***J. Mol. Biol.*** 243:802-805. Berry et al 1995 ***Acta Cryst.*** **D51: 235-239.** Lee et al (1995) ***J. Mol. Biol. 252.*** 15-19.

2. Xia et al 1996 ***IUCr XVII Congress and General Assembly*** C-143. Yu et al (1996) ***BBA*** 1275 47-53. Kim, et al 1996 ***IUCr XVII Congress and General Assembly*** C-144.