ESRF	Experiment title: Test of microcrystals of bacterial bcl complex	Experiment number: LS-687
Beamline: ID-13	Date of experiment: from: 27.4.97 to: 28.4.97	Date of report: 20.10.97
Shifts: 3	Local contact(s): C. Riekel, S. Cusack	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Guillermo Montoya*, Simon Rogers*, Irmi Sinning*

EMBL, Meyerhofstr.1 , D-69012 Heidelberg, Gerrnany

Report:

The **cytochrome bcl complex** plays a key role in respiration in eucary<otic systems as well as in denitrification, cyclic photosynthetic electron transport systems, nitrogen fixation and respiration in bacteria. In mitochondria (complexIII), the bcl complex is involved in transporting electrons and protons across the membrane with a reduced quinone acting and the electron donor and cytochrome c as electron acceptor. In bacteria, the bcl complex consists of only 3 protein subunits which are conserved in higher **organisms:** cytochrome b, cytochrome cl **and** Rieske protein. The bacterial complex has been studied in detail using molecular biology and various spectroscopic techniques, However, the stability of the bacterial bcl complexes studied so far has not been high enoaugh to allow crystallization. Recently, several groups have reported the crystallization of the bcl complex from mitochondria of bovine and chicken. This complex consists of 11 subunits and is not amenable for genetic manipulation. The group of II. Deisenhofer has published preliminary results from the structure determination of bovine bcl complex. However, the crystals suffer from disorder of the Rieske Protein and part of the cytochrome1.

We have been focussing on the preparation of a stable bcl complex from bacteria and have obtained microcrystals. Even when the colour of the crystals is brown (due to cytochrome), one cannot be 100% sure that the crystals really contain the bcl complex. The crystals grow at 4°C under slightly different conditions and

with two different detergents: dodecyl-maltoside and decyl-maltoside. The first ones were tried at the microfocus beamline since they *were* of more hexagonal shape (the other ones are more needle-like). The crystals didn't show really sharp edges. The size of the crystals was around 0.020 X0.015 mm. There was no possibility to test them at home or to optimize the freezing conditions. Some of the crystals suffered during the trip to Grenoble and were lost. It seems like the temperature in the container was too low for them. Only three drops with crystals survived the transport.
The beamline was equipped at that time with a Mar CCD, camera of 133 m m diameter. At the beginning a careful alignment of the beam was carried out with the help of Ch, Riekel. Since the space at ID13 is very limitted and w c wanted to get familiar with the settings, we started with crystals of a soluble protein (NG-domain of FtsY, the SRP receptor from E. coli). These crystals diffracted to about 1.6A. We then started with the bcl complex. For about 8 h we tried many crystals, but no diffraction could be observed. We again tried the FtsY crystals and no diffraction could be observed either, which told us that we had somehow mislaligned the beam- After realigning we continued to test bcl crystals, but again we couldn't observe any diffraction. Improvement of the crystals is clearly needed before the project **can go ahead.** We were quite unlucky that we lost our best samples during the trip and that we lost a lot of time since the beam was misaligned. On the other band, we are now sure that our crystals are not salt.

After slightly more than 2 shifts we had used all our bc1 crystals and then we swapped to new crystals of a mutant of FtsY.
These crystals were needle like (0.1 x 0.2 X 0.050 mm) and they diffracted around 1.6 **A,** The exposure time was 2 seconds. The crystal lasted in the beam for only about 10 to 15 degrees (oscillation rate=l°). When decay was observed in the diffraction pattern the sample was translated in the longest axis and a new region was irradiated. We collected about 80" in total. We couldn't start to process the data while we were at the ESRF since the system was not installed at the time (Mike Blum was still there, setting-up the CCD). Processing these data at home proved to be impossible due to high mosaicity and bad quality of the data (probabely due to radiation damage).
In the meantime, we have obtained better (bigger) crystals of that mutant and could collect **data** on BM14 (see report LS-688).

We had problems at the beginning of our beamtime with the alignment of the beam Is very difficult to position the crystals on the goniometer head without touching some of the elements like the beamstop, collimator etc. We think that improvements are needed in order to make the crystal mounting process more user-friendly. On the other hand the detector and the beam were excellent. Many projects were stopped before because crystals with a reasonable size couldn't be obtained (usually the most interesting ones). The existence of this beamline will certainly boost these projects.