ESRF	**Experiment title:** The crystal structure of the Colicin E3 RNase domain complex with Im3	**Experiment number**: LS-1456
Beamline: ID14.2 & BM14	**Date of experiment**: from: 7th December 1999 to: 8th December 1999 (ID14.2) and from: 9th December 1999 to: 11th December 1999 (BM14)	**Date of report**: 31st March 2000
Shifts: 9 (3+6)	**Local contact(s)**: Stephanie Monaco & Gordon Leonard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr.Andrew Hemmings

Schools of Biological and Chemical Sciences
University of East Anglia
Norwich NR4 7TJ
UK

Report:

This report describes the work carried out on beamlines ID14.2 and BM14 over 3 days in December 1999. The original aim of the experiment was to pursue high resolution and ligand complex studies of the S.frigidimarina ferric reductase. Due to sample problems with this enzyme these objectives became untenable and it was necessary to turn to secondary projects.

(1) The crystal structure of the Colicin E3 RNase domain complex with Im3

Background. Colicins are a diverse family of plasmid-encoded protein antibiotics synthesised by bacteria at times of nutrient or environmental stress as a means of reducing competition from other microbial populations. The RNase colicins such as the colicin E3 are coexpressed along with a small inhibitor protein (Im3) which binds with high affinity, completely protecting the producing cell against the cytotoxic effects of the colicin. We have prepared crystals of a C47M mutant of the E3 RNase domain:Im3 complex labelled with selenomethionine. A 3-wavelength Se K-edge MAD experiment was carried out on BM14.

Results. All data collection was performed at 100 K using a single cryoprotected crystal. The space group was $P3_121$, cell parameters a=93.7 Å, b=93.7 Å, c=76.2 Å with one molecule of complex in the asymmetric unit. X-ray diffraction data were collected from a mutant C47M Im3-containing complex at three wavelengths around the selenium K-edge on station BM14. Images were processed to 2.8 Å resolution. An

additional data set had been collected from a single crystal of the native complex elsewhere and processed to 2.4 Å. Scaling of the MAD data was performed and the positions of the two selenium atoms were found using SOLVE. The resultant map had a figure of merit of 0.55 to 2.8 Å. Phase improvement by solvent flattening was performed using DM at a nominal solvent content of 72%. This produce a phase set with a free R-factor of 29.6%. The initial electron density map was of exceptional quality and displayed clear solvent boundaries for the complex, and it was possible to trace all 84 residues of the immunity protein and residues 2-94 in the RNase domain (Phe 455-Lys 549) using O. Refinement converged to produce a final model with R_{cryst} = 19.2% (R_{free} =22.8%) for all data (applying a 2σ cut-off) in the resolution range 30-2.4 Å.

(2) Enzymes of the bacterial sulfur oxidising pathway - SoxAX

Background. The biogeochemical sulfur cycle involves the continual interconversion of inorganic sulfur compounds between different oxidation states as well as conversion to and from organic forms. The oxidative half of the sulfur cycle in which the environmentally most abundant reduced species, sulfide and thiosulfate, are converted to sulfate is primarily bacterially mediated. Our approach to understanding the molecular basis of sulfur metabolism is through the study of the structures of enzymes of the sulfur oxidation pathway of a model organism, the photosynthetic sulfur-oxidising bacterium, *Rhodovulum sulfidophilum.* We have purified and crystallised a periplasmic inducible thiosulfate-oxidising c-type cytochrome, the SoxAX complex. A native diffraction dataset was collected on ID14.2 and an iron fluorescence scan measured on BM14.

Results. The 3-heme 45kDa SoxAX complex was crystallized using the hanging drop vapour diffusion method to give two suitable crystal forms. A single crystal of the type I form of dimensions 200 x 100 x 20 μm^3 was cryoprotected using ethylene glycol and a native diffraction dataset collected to 1.7Å resolution using oscillations of 1º with 20 second exposures on station ID14.2. Data collection was performed using high and low resolution passes and processed to give an essentially complete dataset (96.1%) with an R_{sym} of 7.0%. The space group is $P2_12_12_1$ and cell dimensions a=66.4Å b=71.7Å c=87.3Å . The crystal was subsequently retrieved and remounted on BM14 where an Fe K-edge X-ray fluorescence scan was performed. The quality of the spectrum suggests that this crystal form will be suitable for an Fe-MAD phasing experiment. In addition, a dataset to 3.0A resolution was collected at a wavelength of 1.74Å close to the iron fluorescence peak (R_{sym} = 6.1%). A number of other crystals of type I SoxAX were soaked in various heavy atom reagents and screening for suitable derivatives was performed on ID14.2. No suitable derivatives were identified in the time available.

(3) Miscellaneous

1. A dataset extending to 1.6Å resolution was collected from the E41A muant of the colicin E9 DNase domian complex with Im9 (R_{sym}=5.1%). Space group $P2_12_12_1$. Cell a=45Å b=53Å c=88Å.

2. Crystals of six other proteins were screened for diffraction quality on ID14.2 during our visit.