| ESRF | **Experiment title: High resolution structure of a mono-oxygenase from Streptomyces coelicolor: a very small enzyme involved in the synthesis of polyketide antibiotics.** | **Experiment number**: LS - 1498 |
|---|---|---|
| **Beamline**: | **Date of experiment**: from: 16-Sep-99 7:00 to: 17-Sep-99 7:00 | **Date of report**: 15 Feb 2000 |
| **Shifts:** | **Local contact(s)**: Dr. Ed .Mitchell | *Received at ESRF:* |

**Names and affiliations of applicants** (* indicates experimentalists):

**Dr. Beatrice Vallone** Dip. Di Scienze Biochimiche – Università di roma "La Sapienza" – P.le Aldo Moro, 5 – 00185Roma- Italy **Vallone@axcasp.caspur.it**

**Dr. Steven Kendrew** Dip. Di Scienze Biochimiche – Università di roma "La Sapienza" – P.le Aldo Moro, 5 – 00185Roma- Italy

**Dr. Adriana E.** Miele Dip. Di Scienze Biochimiche – Università di Roma "La Sapienza" – P.le Aldo Moro, 5 – 00185-Roma- Italy

**Report**: The aim of the experiment was to obtain the high resolution structure of a mono-oxygenase from the biosynthetic pathway of the antibiotic actinorhodin, which could provide information relevant in the design of new antibiotics and clarify the mechanism of catalysis that allows the insertion of an oxygen atom into an inorganic molecule without the use of a cofactor. Since no homologous structure is known we decided to solve the structure of ths protein by MIR

We needed to use synchrotron radiation since, in spite of our early efforts, the crystals did not grow to a size that could allow in house high resolution data collection.

During the experiment carried out at ESRF at beam line ID 14-2 we have attempted to collect:

° A high resolution data set.

° Data from crystals soaked in different heavy atom salts.

° Data from crystals exposed to high Xenon pressure using the Xenon cell available at ESRF.

The first task was successful since we have collected, using one of the very few large crystals which sometimes we manage to obtain, a complete data set (99.9%) at 1.4 A resolution of high quality (Rmerge= 8%, $I/\sigma$ =17.2 ) and we could observe diffraction up to 1.2 A, indicating that using one of the very few large crystals and setting up a targeted experiment it could be possible to collect data at atomic resolution.

As for the production of derivatives to phase the native data the experiment has not been successful, indeed we have used all the available time and collected 8 data sets for 5 different soaks at resolutions ranging from 1.8 A to 2.2 A(Sm, Gd, Ir, Au and Pt).

Subsequent data analysis showed that in all cases poor or no derivatives were obtained. This finding, in conjunction with the experiment we have already carried out at home using mercurials, uranium and lead, indicate that our crystals are not reactive with respect to the reagents currently used for the production of isomorphous derivatives (probably because of the small size of the monoxygenase and of the tight crystal packing).

A more extensive search of heavy atom derivatives is hindered by difficulties in obtaining several crystals large enough to be tested on conventional x-ray sources.

The attempts to produce a Xenon derivative failed since the cell available at ESRF is probably optimized for crystals grown in low salt conditions and ours, wich are produced in 1.5 M ammonium sulphate, dried during the exposure to 10 Atm. Of Xe.

We are presently testing the Oxford Cryosystems Xe-Cell and preliminary results indicate that with a smaller volume and equilibration of the Xe gas with mother liquor indeed it would be possible to try to probuce a xenon derivative.

The learned assistence provided by the beam line scientist has been essential to obtain the best quality of data and optimal use of the assigned time, we wish to thank him for that and for the help he gave us to recover our data from DAT tapes due to failure of our home unit.