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Report:

LS-779: Structural Analysis of Colicin E9 DNase:Immunity Protein Interactions

The following report details the results of visits to ESRF-BM14 on 29th to 31st August 1997 and a return visit on 10th to 12th December 1997. The second visit was arranged because of severe technical problems with station hardware during the August visit. The original proposal requested beamtime for a selenium MAD experiment to solve the structure of a Colicin E9 DNase:Immunity protein complex. However, in the months before the visit it was possible using the program SHARP to calculate phases using MAD data collected previously on these crystals at Hamburg. With the permission of the station manager, crystals of an alternative protein (isoflavocytochrome c from *Shewanella Putrefaciens*) were brought to attempt an iron MAD experiment. This work is described below.

Crystal structure analysis of the isoflavocytochrome c from Shewanella Putrefaciens

Shewanella putrefaciens is a Gram-negative, facultative aerobe which produces several c-type cytochromes under anaerobic conditions. The most abundant of these is flavocytochrome c which has been shown to catalyze fumarate reduction. Fumarate is a terminal electron acceptor in the anaerobic respiratory pathways of many facultative anaerobes. The fumarate reductase of *E.coli* and other bacteria is a membrane-bound

enzyme of four subunits, the largest being a 69kDa protein containing covalently bound flavin, whereas the soluble, periplasmic flavocytochrome *c* of *Sputrefaciens* contains non-covalently bound FAD.
Recently, a periplasmic enzyme has been purified from *S.putrefaciens* which is induced in the presence of iron (III) citrate which has fumarate reductase activity and which has significant sequence homology to the previously characterized flavocytochrome c (Paul Dobbin, personal communication). This enzyme has been termed isoflavocytochrome c. The enzyme has a molecular weight of approx. 64kDa and contains four c-type heme groups and an FAD unit. There is no sequence similarity to any protein of known structure.
We have crystallized this enzyme using hanging drop vapour diffusion in drops containing equal volumes of protein solution and 12% PEG 20K buffered with sodium HEPES pH 7.5. Crystals appear after 16 hours at 18°C and grow to dimensions of approximately 0.25 x 0.1 x 0.1 mm^3. These crystals can be cryoprotected by sequentially soaking for 2 minutes in solutions containing 12% PEG 20K and 15%, 20% and finally 25% ethylene glycol.

The data collected during our visits to ESRF-BM14 are summarised below:
(1) 29th to 31st August 1997
On the first visit we were collected a native diffraction dataset with the following statistics:

Wavelength/A	Resolution/Å	Rmerge/%	Reflections	Completeness/%
1.0355	30-3.0	5.6	35 842	99.4

Self-rotation functions have been used to try to identify non-crystallographic symmetry elements, however none are readily detectable from the native diffraction data.

(2) 10th to 12th December 1997
During the second visit, only 5 shifts were available. This was insufficient time to allow us to get the best quality data but the following datasets were collected at three wavelengths around the iron edge from a single crystal.

Wavelength/A	Resolution/A	Rmerge/%	Reflections	Completeness/%
1.7341	30-3.5	8.3	23 073	96.2
1.7399	30.3.75	7.6	16 969	89.1
1.0355	30-3.5	5.6	17 235	71.8

It has not been possible to unambiguously identify any of the iron sites from this data. Given the crystal cell dimensions and the known molecular weight, there may be up to sixteen haems in the asymmetric unit. A larger crystal is required to collect reliable data to higher resolution about the iron edge if we are to be able to locate their positions in the cell.