ESRF

Experiment title: MhpC: a C - C bond hydrolase	**Experiment number:** LS-1533

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Shifts: 3	**Local contact(s):** Gordon Leonard	*Received at ESRF:*

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Report:

MhpC is a C-C bond hydrolase involved in the bacterial degradation of phenylpropionic acid. Hydrolytic cleavage of a C-C bond is a rare enzymic event in nature and yet it is a key step in the extradiol cleavage pathways for bacterial degradation of aromatic compounds. MhpC may have potential in biotransformation reactions. The enzyme is also of interest in terms of engineering organisms capable of removing persistent toxic compounds from the environment.

We have managed to grow crystals of MhpC (kindly provided by Professor T. Bugg, Warwick) which diffract to high resolution (~ 2.2 Å at 100K). The enzyme shares very weak sequence identity with functionally similar enzymes from other organisms but molecular replacement using several such structures has not been successful so far. Thus we initiated a screen for heavy atom derivatives but this proved unsuccessful partly due to problems of non-isomorphism of the heavy atom soaks and co-crystals. We have since expressed selenomethionine-substituted enzyme which yielded suitable crystals.

MAD data were collected on a single crystal of Se-Met MhpC at BM14 (ESRF) using the MARCCD detector. The three datasets each of 120 degrees were collected at the selenium edge using 0.5 degree oscillations of 60 seconds per image. The data were processed using DENZO (A. Brunger) which indicated something was wrong with our initial choice of an orthorhombic space group. Reprocessing in P1 and inspection of the data showed the space group to be $P2_1$ with 4 subunits in the asymmetric unit. Reprocessing in $P2_1$ gave far improved statistics: R_{merge} values for the f'' max dataset, f' min and remote datasets were 2.9 %, 3.2 % and 3.0 %, respectively at 2.3 Å.

Since the enzyme has a large number of selenium sites in the asymmetric unit (~ 40) we opted for the direct methods Shake and Bake approach (C. Weeks) to locate the anomalous scatterers. This produced a solution which contained 32 sites. Inspection of these indicated that there were 4 discrete groups of 8 sites related by NCS operators. Initially phasing was performed using MLPHARE and DM (CCP4 suite) which strongly indicated that the solution was correct although the resulting maps were difficult to interpret. However a much improved map was obtained by phasing with CNS (A. Brunger). The sequence is now being fitted. Samples of the electron density at 2.5 Å from the CNS phasing are shown below.

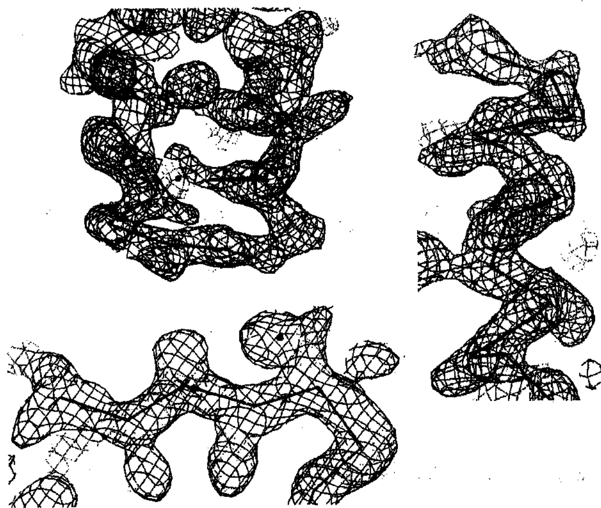

Figure 1 (above). Samples of electron density at 2.5 Å for the MhpC enzyme obtained by MAD phasing. The dark spheres indicate selenium sites.

We are now in a position to collect high resolution data on the enzyme to aid refinement of the structure. We will also collect data on inhibitor complexes and eventually site-directed mutants.