ESRF	**Experiment title:** The Structure of *Neurospora crassa* 3-Carboxy-*cis,cis*-Muconate Lactonising Enzyme	**Experiment number**: LS-1102
Beamline: BM-14	**Date of experiment**: from: 4.12.1998 to: 6.12.1998	**Date of report**: 25.2.2000
Shifts: 6	**Local contact(s)**: Dr. Andrew Thompson	*Received at ESRF:*

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Report:

Neurospora crassa 3-carboxy-*cis,cis*-muconate lactonising enzyme (CMLE) is an enzyme of the β-ketoadipate pathway in soil microbes, that convers aromatics such as lignin to citric acid cycle intermediates. There are three evolutionary unrelated classes muconate lactonising enzymes (MLEs), that differ in their catalytic requirements and stereochemistry (*syn* or *anti*-addition), but catalyse the same reaction. The eucaryotic MLEs form a class of their own, of which *Neurospora crassa* CMLE is an example. Therefore the MLEs provide an interesting case of convergent functional evolution. Also the eucaryotic enzymes show no significant sequence similarity to any known structure in the PDB.

A Full three wavelength MAD-experiment was carried out on the of SeMet-substituted form of *Neurospora crassa* 3-carboxy-*cis,cis*-muconate lactonising enzyme (CMLE) at the BM-14 beam line. Semet-CMLE crystallised as the native protein, from 0.1 M PIPES pH 5.7, 1.56 M $(NH4)_2SO_4$, with added 5 mM β-mercaptoethanol. Mass-spectrometry showed 470 a.m.u. difference in M_w to native, indicating full substitution by selenomethionine. CMLE is a homotetrameric (4x40 kDa) enzyme with 10 methionines per monomer and crystallises in $P2_12_12_1$ with two tetramers in an asymmetric unit (Glumoff *et al*. 1996), providing a technically challenging experiment as the content of the asymmetric unit of the crystal mount to 320 kDa and 80 Selenium positions to be found.

CMLE was flash frozen from 40% PEG 400, and as PEG 400 is immiscible with large concentrations of $(NH4)_2SO_4$, the flash cooling of CMLE crystals has been problematic as the exchange from high salt to 40% PEG 400 (being the best choice) stresses the crystals,

causing an increase in the mosaicity and resulting in highly disordered crystals, often with mosaicity close to 1.0°, therefore limiting the useful data resolution to lower than 3 Å at best, although the crystals diffract to ~2 Å. Data sets at three wavelenthgs (the first, Se-edge, was complete to 3 Å) (Table 1) were collected from a crystal with slightly altered cell dimensions ($P2_12_12_1$; a=92.38, b=152.13, c=247.14) from the published 'dominant' crystal from (Glumoff *et al*. 1996).

The selenium sites were found by Shake'n'Bake v. 2.0 (Miller & Weeks 1998) during the EMBO MAD workshop, March 1999 in Grenoble, 60 sites of the total 80 excepted were found using the anomalous diffresences of the peak wavelength (SAS data) at 4 Å resolution. The structure is one of the largest SeMet-structures solved to date along with the ADP-L-mannoheptose 6-epimerase (Deacon and Ealick 1999).

The Se-sites were used as input to MLPHARE (Otwinowski 1991) to obtain phases (Table 2). After the NCS-operators for the twofolds of the two (D_2) tetramers in the asymmetric unit were found, solvent flattening, 4-fold NCS avering and and phase extention to 3.0 Å were performed using DM (Cowtan 1994). This yielded readily interpretable electron density maps into which the molecule was build. The maps revealed that *Nc*CMLE has a β-propeller fold, completely different from the structures of other classes of MLEs (bacterial MLEs and CMLEs) (Helin *et al.* 1995). Molecular replacement into crystal form with higher resolution data (Glumoff *et al*. 1996) has been done and the refinement of the structure is in progress.

Table 1. Data collection statistics.

Dataset	λ = 0.9786 Å (peak)	λ = 0.9795 Å (inflection point)	λ = 0.8856 Å (remote)
Resolution	20-3.0 Å	20-3.2 Å	20-3.5 Å
Completeness	92 % (71.3%)*	83 %	75%
R_{sym}	6.3 %	4.3 %	4.3%
I/σ	12.8 (6.0)*	17.5	22.9

*****Highest resolution shell.

Table 2. Phasing statistics.

	MLPHARE (20-4Å)
Figure of merit	0.55
R_{cullis} (acentric/centric/anom.)	0.62 / 0.54 / 0.72
Phasing power (acentric/centric)	2.00 / 1.52