ESRF	**Experiment title:** MAD Data Collection on 5-ALA-dehydratase	**Experiment number:** LS400
Beamline: BM14	**Date of experiment:** from: 25th Sept. 1996 to: 26th Sept. 1996	**Date of report:** Feb 1997
Shifts: 6	**Local contact(s):** Dr. A. Thompson	*Received at ESRF:* 17 FEB 1997

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Introduction

The X-ray structure of the enzyme 5-aminolaevulinate dehydratase (porphobilinogen synthase) from yeast has been solved to a resolution of 2.3 A using MAD data collected on the ESRF beamline BM14. The enzyme (abbreviated ALAD) catalyses an early key step in the biosynthesis of porphyrins and corrins namely the condensation of two 5-aminolaevulinic acid (ALA) molecules to form the pyrrole porphobilinogen (Jaffe 1995, Jordan 1994). Hereditary deficiencies of ALAD give rise to acute porphyria and the enzyme is highly sensitive to inhibition by lead ions. In both these conditions the accumulation of the substrate, which mimics the neurotransmitter GABA, is thought to provoke the associated neurological symptoms. ALAD also appears to be a regulatory component of the mammalian 26S proteasome.

MAD data collection at BM14

We have expressed selenomethionyl yeast ALAD and obtained crystals which grew in space group 1422 with cell dimensions of a=b=102.8 A c=167.9A and have one monomer per asymmetric unit with an estimated solvent content of 59%. The crystals were cryoprotected for data collection at 100 K by slow addition of glycerol to 30% v/v and flashed cooled by immersion in liquid ethane prior to storage under liquid nitrogen. Inverse beam selenium MAD data to 2.4 A resolution were collected from a cryocooled crystal at beamline. Image plate data (Maresearch) were collected at wavelengths corresponding to the maxima of f ' and f " determined from an in-situ XANES scan. In addition, a reference dataset was collected from the same crystal at a lower wavelength and a high resolution dataset (2.1 A) from another selenomethionyl ALAD crystal was obtained. These data were processed with the program MOSFLM and the CCP4 suite (1994). Since the crystals were randomly oriented, anomalous differences were calculated in AGROVATA from all measurements of each hkl and the optimised f ' and f " data were scaled anisotropically to the reference dataset with SCALEIT. Details of the data collection and processing are given in the following table.

Dataset	Max f'	Maxf"	Reference	High Resolution
λ (Å)	0.97934	0.97913	0.9537	0.97913
Resolution (A)	2.45	2.45	2.40	2.1
Rmerge (%)	5.0	5.1	4.9	9.3
Outer shell Rmerge (%)	16.1	14.2	15.6	27.6
% I > 3 σ(I)	92.1	92.1	91.5	86.7
% $\|\Delta_{ano}\| > 3\ \sigma(\Delta_{ano})$	5.1	19.0	8.7	5.7
Mean multiplicity	9.6	9.5	9.4	15.0
Completeness (%)	99.4	99.4	99.5	99.9

Phasing and refinement

A total of 5 selenium sites were located with the optimised f " dataset using an anomalous difference Patterson which was interpreted with the vector search and refinement programs VECSUM and VECREF. Phases to 2.5 A were calculated using the program MLPHARE in which the optimised f ' and f " data were treated essentially as derivative datasets. Maps were calculated with the selenium sites on both possible hands, ie (x,y,x) and (-x,-y,-z). The latter was found to produce a map which had strong signs of a protein/solvent boundary thereby defining the absolute hand of the selenium constellation. Solvent flattening and histogram matching were then carried out using DM which yielded an initial map of excellent quality allowing 93% of the structure to be modelled at that stage. Least-squares restrained refinement has been performed using the program RESTRAIN (Haneef et al., 1985) between rounds of rebuilding which have lowered the R-factor to around 22% at 2.3 A resolution.

X-ray structure

The enzyme forms a large homo-octameric structure with 422 (or D_4) symmetry and gross dimensions of 104 x 104 x 94 A^3. Each subunit has a molecular weight of around 38 kDa and adopts an α/β fold with an extended arm which forms numerous intersubunit interactions. In the active site of each subunit there are two lysine residues (210 and 263) and one of these, Lys 263, is known to form a Schiff base link to substrate. The two lysine side chains are close to a zinc ion which is bound by cysteine residues and may have an important structural and/or catalytic role.

Acknowledgements

We gratefully acknowledge Dr Andy Thompson (European Synchrotron Radiation Facility (ESRF) and the European Molecular Biology Laboratory (EMBL) for invaluable assistance with the MAD measurements. We thank N. Senior and M. Warren (Institute of Ophthalmology, University of London) for the selenomethionyl and wild-type yeast ALAD.

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