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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title: Structural studies on 3-methyl aspartate ammonia lyase**	**Experiment number**: LS 1590
Beamline: BM30A	**Date of experiment**: from: 5 May 2000 to: 7 May 2000	**Date of report**: 22-8-01
Shifts: 6	**Local contact(s)**: Michel Roth /Eric Fanchon	*Received at ESRF:*

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Report:

Methylaspartate ammonia lyase (MAL) catalyses the magnesium-dependent reversible α,β-elimination of ammonia from L-*threo*-(2S,3S)-3-methylaspartic acid to give mesaconic acid. This enzyme has considerable biotechnological potential for the chiral synthesis of novel amino acids but, currently, its three dimensional structure is unknown, as is the molecular basis of substrate specificity and catalysis.

Data were collected to 2.1Å resolution at three wavelengths (0.979628 Å, 0.979463 Å & 0.968634 Å) from a single cryo-frozen Se-Met form A crystal at station BM 30A of the ESRF, using the inverse beam geometry rotation camera method and a MAR345 image plate. The wavelengths corresponded to the Se K-edge f' minimum, f" maximum and high energy remote positions, as determined on the basis of an X-ray fluorescence spectrum collected directly from the crystal, which was analysed by the program CHOOCH (Evans, G. 1994). A solution for all 10 of the selenium atoms in a single polypeptide chain was found in space group $P4_122$ using the program SOLVE.

To produce an atomic model of MAL automatic model building was performed with the program ARP/wARP 5.0 (Perrakis, A., 1999) using the phases output from SOLVE at 2.1 Å. The warpNtrace procedure within ARP/wARP 5.0 produced a partial model containing 380 of the 413 residues with a mixture of fully and partially built side chains. The remaining parts of the main chain structure were built manually using O (Jones

et al., 1991), side chains were not rebuilt at this point. This model was then subjected to the side-chain identification and sequence docking procedure associated with warpNtrace (Perrakis *et al*., 1999). The sequence was docked with 100% certainty and the resulting model was subjected to several rounds of rebuilding and refinement with REFMAC (Murshudov *et al*., 1997) using data in the resolution range 20 Å- 2.1 Å. Water molecules were added with the program ARP (Lamzin *et al*., 1997) which was run in conjunction with REFMAC**.**

Each subunit of MAL comprises two domains, one of which adopts a folding pattern reminiscent of the triose phosphate isomerase (TIM) barrel. Substrate binding studies have identified the active site as lying at the C-terminal end of the barrel. Structure comparisons have shown that, despite very low sequence similarity, the structure of MAL is closely related to representative members of the enolase superfamily including Mandelate Racemase, Muconate Lactonising Enzyme I and Enolase. On the basis of its structure, the mechanism of MAL is clearly related to that of the enolase superfamily and involves the initial abstraction of a proton α to the 3-carboxyl of (2S,3S)-3-methylasparic acid, to yield an enolic intermediate. Key residues involved in catalysis are a cluster of 3 carboxyls that bind the Mg^{2+} (Asp 307, Glu 273 & Asp 238) and a base (Lys 331) to abstract the proton. This analysis resolves the conflict which had linked MAL to the histidine and phenylalanine ammonia lyase family of enzymes, which require a post-translationally modified residue at the active site and for which the structure of MAL provides no support.