EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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 original report should be sent, together with 5 reduced (A4) copies, to the User Office.

In addition, please send a copy of your file as an e-mail attachment to reports@esrf.fr**,** using the number of your experiment to name your file. This will enable us to process your report for the ESRF Annual Report.

Reports accompanying requests for additional beam time

If your report is to support **a new proposal**, the orginal report form should be sent with the new proposal form, and a copy of your report should be attached to each copy of your proposal. 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.
- bear in mind that the report will be reduced to 71% of its original size. A type-face such as "Times", 14 points, with a 1.5 line spacing between lines for the text, produces a report which can be read easily.

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ESRF	**Experiment title:** **Structural studies on KDPG-aldolase from *Thermotoga maritinum***	**Experiment number**: LS-1951
Beamline: ID14EH2	**Date of experiment**: from: 03.06.01 to: 04.06.01	**Date of report**: 21.08.01
Shifts: 3	**Local contact(s)**: Dr. S. Kozielski	*Received at ESRF:*

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Aldolase

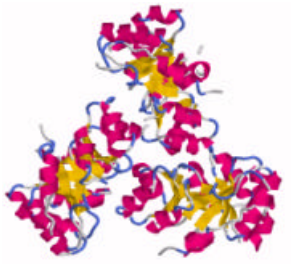
As part of our program to probe the mechanism of the aldolase and to direct evolution of novel substrate profiles of KDPG aldolase we collected the following data

KDPG-aldolase from *Thermotoga maritinum* crystallises in space-group $P2_1$ with two trimeric molecules in the assymetric unit. The protein is from a thermophile thus making it more useful for synthetic use. The monoclinic angle has a value of 92º, which made the symmetry of these crystals not obvious at first. This was our first attempt to collect diffraction data of this protein, so the space-group and conditions for cryo-protection were not known at the start of this diffraction experiment. Luckily, cryo-protection could easily be achieved by briefly washing crystals in a mixture of 8 µl of mother liquor from the crystal plate with 2 µl of 75 % (v/v) PEG 600. The crystals diffracted to a resolution of 2.2 Å, but unfortunately the exposure time had to be reduced in the middle of the experiment to to time constraints. The data given in Table 1 is complete to 3.0 Å.. The structure has been solved using the molecular replacement programs AMORE and BEAST with the trimeric KDPG-aldolase from E. coli (PDB-entry 1EUA). This protein has a sequence identity of approx. 30 % and molecular replacement thus did not give very clear solutions. Refinement of the structure was greatly aided by exploiting the sixfold noncrystallographic symmetry of the assymetric unit. A ribbon representation of the KDPG-aldolase trimer is shown in Fig 1.

Data collection	**KDPG-aldolase**
Wavelength (Å)	0.933
Resolution (Highest Shell, Å)	52.71 – 2.20 (2.21 – 2.20)
Space group	$P2_1$
Cell constants (Å; º)	a=43.9, b=100.5, c=125.1; a=?=90; ß=92.1
Total measurements	114186
Unique reflections	26676
Average redundancy	3.3 (1.3)
I/s	5.4 (2.0)
Completeness (%)	69.3 (50.5)
R_{merge} [b]	10.6 (35.7)

[a]R_{merge} = SS $I(h)_j$ - <I(h)> / SS $I(h)_j$ where $I(h)_j$ is the measured diffraction intensity and the summation includes all observations.

Figure 1: Ribbon representation of KDPG-aldolase from *Thermotoga maritinum*



Crystals of 2 mutants of KDPG aldolase from *E.coli* were brought to Grenoble in sitting drop plates. Crystal diffraction quality varies considerably across any given set of crystals therefore a large number of crystals were screened before one of suitable quality and resolution was found.
One crystal of a multiple mutant from Professor Wong's labaoratory did diffract. The multiple mutant is of interest because it has a reversed sterochemistry of substrate addition. The crystal measured (0.7 mm x 0.25 mm x 0.20 mm) and diffracted to 2.3Å. A complete dataset was collected on the crystal consisting of 342 frames, the crystal having been exposed for 30s per frame at a distance of 150 mm.
The data was processed onsite with Molsflm and scaled with Scala. Details in Table 2 below

Temperature	110 K
% completeness	98%
No. reflections	28060
Resolution	2.3Å
Space group	C2
Cell	161x122x88

Data was molecular replaced with aMoRe using native KDPG aldolase (from *E.coli*) as a model. This gave a solution which had a trimer in the asymmetric unit which is consistent with other data. The data is being refined with Refmac5 and currently has an Rfree of 26%.

Methylaspartase
3-Methylaspartase (ß-methylaspartase, BMA; E.C. 4.3.1.2) catalyses the reversible *anti*-elimination of ammonia from L-*threo*-(2S,3S)-3-methylaspartic acid to give mesaconic acid. The reverse reaction is of particular interest because it can be exploited to generate highly functionalised L-amino acids.
As 3-methylaspartase from *Clostridium tetanomorphum* shows only low sequence relationship to known structures from the Protein Data Bank, the structure was solved by exploiting the anomalous signal of Se-substituted crystals using data collected at the peak wavelength on BM14 in another experiment. In addition we collected a native data set to 1.9 Å on ID14EH2 with the statistics given below in Table 3.
The structure has in the meantime been refined using a hybrid warpNtrace/manual model building approach. A ribbon representation is shown in Figure 2.

Data collection	**BMA-native**
Wavelength (Å)	0.933
Resolution (Highest Shell, Å)	30.86 – 1.90 (1.95 – 1.90)
Space group	$P2_12_12_1$
Cell constants (Å; º)	a=67.3, b=109.3, c=109.0; a=ß=?=90
V_M	2.19
Total measurements	248183
Unique reflections	62524
Average redundancy	3.9 (3.7)
I/s	6.1 (1.9)
Completeness (%)	94.2 (81.2)
R_{merge}[b]	9.4 (39.8)

Structure of methylaspartase