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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:** Structure-function studies of enzymes involved in nucleotide metabolism	**Experiment number**: LS-2012
Beamline: ID14-1	**Date of experiment**: from: 8th february 2002 to: 9th of February 2002	**Date of report**: 26/8-2002
Shifts: 0 (with ls-2029)	**Local contact(s)**: Elena MICOSSI	*Received at ESRF:*

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Report:

Orotidine 5'-monophosphate decarboxylase

Orotidine 5'-monophosphate decarboxylase (ODCase) catalyses the decarboxylation of orotidine 5'-monophosphate (OMP) to uridine 5'-monophosphate (UMP). ODCase is one of the most proficient enzymes known. At ambient temperatures it enhances the rate of reaction by a factor of 10^{17} [1]. Considering the extreme proficiency, the mechanism of ODCase is unique, because no cofactor is required for the decarboxylation.

The structure of ODCase from *E. coli* in complex with a very potent inhibitor [2,3] revealed the importance of a catalytic Lys-Asp-Lys-Asp array which must be essential for decarboxylation. Comparison with the apo-enzyme of ODCase from *E. coli* [4] suggested that also other conserved residues could be essential for catalysis.

We have investigated several important mutant enzymes kinetically, and have crystallized a completely inactive variant of ODCase, where we believe that the necessary conformational changes of ODCase are blocked.

The very thin plate like crystals bended whenever we tried to manipulate them, resulting in a very large mosaicity in certain directions. Although some of the diffraction images had streaks instead of spots, we collected a 360° data set on ID14-2. The crystal diffracted to

2.8 Å. The data could be indexed and merged in C2. We had to fix the mosaicity to 1.5° and got a merging R of 0.157. A molecular replacement solution was found, using the complexed/uncomplexed model with 4 molecules in the asymmetric unit. The exchanged amino acid residues were easily recognised in the electron density map and the model is currently being refined, presently with an R of 0.239 and R_{free} of 0.299. From this model we expect to be able to shed further light on the catalytic machinary of ODCase.

CTP synthase

CTP synthase catalyses the formation of CTP from UTP. We have been able to crystallise CTP synthase from *L. lactis* in monoclinic symmetry with a cell of a=113.7 Å, b=162.1 Å, c=254.4 Å and β=100.8°. The best of these crystals diffracted to 8 Å at ID14-2, which were the same as observed on our in-house equipment, indicating that the crystal quality has to be significantly improved.

Dihydropyrimidinase

Dihydropyrimidinase (DHPase) catalyses the reversible hydrolysis of 5,6-dihydrouracil to N-carbamoyl-ß-alanine, which is the second step in the reductive catabolism of pyrimidine nucleotides. We have been able to crystallise DHPase from *Saccharomyces klyveri* in orthorhombic symmetry with cell dimensions a = 71 Å, b = 101 Å, c = 163 Å. Diffraction to 4 Å were observed for a small crystal (0.05mm x 0.05mm x 0.02mm). A dataset was not collected due to the modest resolution and ice formation.

dCTP deaminase

dCTP deaminase catalyses the formation of dUTP from dCTP. The small (30 x 30 x 30 µm), possibly monoclinic crystals of *E. coli* dCTP deaminase that were tested, difracted to 5.5 Å resolution at ID14-2. This is a big improvement from beamline I711, MAX-Lab where no diffraction from these crystals is seen.

References

1. A. Radzicka and R. Wolfenden, (1995). Science, **267**, 90.
2. P. Harris, J.-C. N. Poulsen, K. F. Jensen and S. Larsen, (2000). Biochemistry, **39**, 4217.
3. J.-C. N. Poulsen, P. Harris, K. F. Jensen and S. Larsen (2001). Acta Crystallogr. D**57**, 1251.
4. P. Harris, J.-C. N. Poulsen, K. F. Jensen and S. Larsen, (2002). J. Mol. Biol. **318**, 1019.