EUROPEAN **S**YNCHROTRON **R**ADIATION **F**ACILITY

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 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:** Structures of inhibitor cmplexes and mutant enzymes of the *E.coli* dihydroorotate dehydrogenase (DHOD).	**Experiment number:** LS−1907
Beamline: ID14−2	**Date of experiment:** from: 26.03.2001 to: 27.03.2001	**Date of report:**
Shifts: 2	**Local contact(s):** Dr. Stephanie Monaco	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Sofie Nørager*

Leila Lo Leggio*

Heidi Aschenfeldt Ernst*

Sine Larsen

Centre for Crystallographic Studies
Department of Chemistry, University of Copenhagen, Universitetsparken 5, Copenhagen, Denmark

Report:

Dihydroorotate dehydrogenases (DHODs) catalyse the oxidation of (S)−dihydroorotate to orotate, the fourth step in the *de novo* biosynthesis of pyrimidine nucleotides. This family of enzymes can be divided into two classes based on their cellular location and on the electron acceptors used *in vivo* [1]. These enzymes have been studied for several years, as they are interesting targets for drugs against cell proliferation. Their use of different electron acceptors and small structural differences has made it possible to inhibit the DHOD from one organism leaving other organisms unaffected. By now structures of representatives of class 1 and class 2 have been solved [2,3,4,5]. To further elucidate the reaction mechanism we have now turned towards mutant studies of either, the *Lactococcus lactis* DHOD A (DHODA), a representative of class 1, or of the *E.coli* DHOD a representative of class 2. A particularity of the class 2 enzymes is their prolonged N−terminus, which folds into 3 helices forming a hydrophobic pocket, the binding site for the electron acceptor. In the case of the human enzyme [4] inhibitors have been shown to bind in this N−terminal. These inhibitors do not affect the *E.coli* enzyme, while other products inhibit the *E.coli* enzyme but not the human DHOD. The availability of a structure of the *E.coli* DHOD complexed with an inhibitor would allow a more detailed comparison of the binding sites.

During the beamtime allocated at the ESRF we collected data on the R57A mutant of *Lactococcus lactis* DHODA. The data was collected to a resolution of 1.7 Å and the data was integrated and scaled using DENZO and SCALEPACK (R_{sym}=4.7%, completeness=99.8%). The structure has been refined in the resolution range 25−1.7 Å (R=17.2% and R_{free}=19.2%) and has together with other mutant structures solved, allowed us to gain insight into the structural features important for enzyme catalysis, active site loop movement and substrate attraction [6].

In addition to this we collected a data set on the *E.coli* DHOD in complex with an inhibitor given to us by the company LICA pharmaceuticals. This data set was collected to a resolution of 2.5 Å and after integration and scaling we obtained a R_{sym}=7.4 % (completeness=99.8%). The asymmetric unit in this crystal contains 8 independent molecules, which complicated the refinement. The structure has been refined in the resolution range 25−2.5 Å with an R=23.1 % and R_{free}=28.1 %. Each of the 8 molecules in the asymmetric unit shows a different degree of inhibitor binding. The two molecules with highest amount of inhibitor bound have allowed us to compare this structure to the human complexes and to give a structural explanation for the different inhibition kinetics observed.
The third data set collected was on the active site mutant of the *E.coli* DHOD (S175C). Unfortunately this data set could only be scaled to 3.1 Å although the resolution limit for data collection was 2.5 Å. It seemed very hard to detect small differences resulting from the mutation at 3.1 Å data, so we decided to try obtain an higher resolution data before refining the structure [7]. Two other data sets were collected but are still in process. These include a 2.1 Å resolution data set of the orotate complexed native *E.coli* and a 2.5 Å data set of the NADH complexed DHOD B from *Lactococcus lactis*.

[1] Björnberg,O., Rowland,P., Larsen,S. & Jensen,K.F. (1997). *Biochemistry* **36**, 16197−16205.
[2] Rowland,P., Bjornberg,O., Nielsen,F.S., Jensen,K.F. & Larsen,S. (1998). *Protein Sci.* **7**, 1269−1279.
[3] Rowland,P., Nørager,S., Jensen,K.F. & Larsen,S. (2000). *Structure.Fold.Des* **8**, 1227−1238.
[4] Liu,S., Neidhardt,E.A., Grossman,T.H., Ocain,T. & Clardy,J. (2000). *Structure.Fold.Des* **8**, 25−33
[5] Nørager,S., Jensen,K.F., Björnberg,O. & Larsen,S. In press
[6] Nørager,S., Arent, S., Ottosen, M., Lo Leggio,L., Jensen,K.F., & Larsen,S. In preparation
[7] Nørager,S., Björnberg,O., Jensen,K.F. & Larsen,S. In preparation