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 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.

<table>
<tr><td> </td><td>Experiment title:
Tromsø Structural Biology Centre - application for block allocation of beamtime</td><td>Experiment number:
01-02-643</td></tr>
<tr><td>Beamline:
BM01A</td><td>Date of experiment:
from: 18.07.03 to: 21.07.03
and 28.11.03 to: 02.12.03</td><td rowspan="2">Date of report:
12.05.06

Received at ESRF:</td></tr>
<tr><td>Shifts:
9 + 12</td><td>Local contact(s):
Dr. Jon Are BEUKES</td></tr>
<tr><td colspan="3">Names and affiliations of applicants (* indicates experimentalists):

Edward Hough, Arne O. Smalås, Ole A. Andersen*, Ronny Helland*, Ellen Wang*
Department of Chemistry
University of Tromsø
N-9037 Tromsø
Norway</td></tr>
</table>

Publications related to this experiment:

1 . Moe, E., Leiros, I., Riise, E.K, Olufsen, M., Lanes, O., Smalås, A. O. & Willassen, N. P. (2004). Optimisation of electrostatic surface potential as strategies for cold adaptation of Uracil DNA glycosylase (UNG) from cod (Gadus morhua). *J. Mol. Biol.*, **343**, 1221-1230.

Cold-adapted enzymes are characterised by an increased catalytic efficiency and reduced temperature stability compared to their mesophilic counterparts. Lately, it has been suggested that an optimisation of the electrostatic surface potential is a strategy for cold adaptation for some enzymes. A visualisation of the electrostatic surface potential of cold-adapted uracil-DNA N-glycosylase (cUNG) from Atlantic cod indicates a more positively charged surface near the active site compared to human UNG (hUNG). In order to investigate the importance of the altered surface potential for the cold-adapted features of cod UNG, six mutants have been characterised and compared to cUNG and hUNG. The cUNG quadruple mutant (V171E, K185V, H250Q and H275Y) and four corresponding single mutants all comprise substitutions of residues present in the human enzyme. A human UNG mutant, E171V, comprises the equivalent residue found in cod UNG. In addition, crystal structures of the single mutants V171E and E171V have been determined. Results from the study show that a more negative electrostatic surface potential reduces the activity and increases the stability of cod UNG, and suggest an optimisation of the surface potential as a strategy for cold-adaptation of this enzyme. Val171 in cod UNG is especially important in this respect.

2. Helland, R., Larsen, A.N., Smalås, A.O. and Willassen, N.P. (2006) The 1.8 Å crystal structure of a Proteinase K like enzyme from a psychrotroph Serratia species. *FEBS J.* ,**273**, 61-71.

Proteins from organisms living in extreme conditions are of particular interest because of their potential for being templates for redesign of enzymes both in biotechnological and other industries. The crystal structure of a proteinase K-like enzyme from a psychrotroph Serratia species has been solved to 1.8 A. The structure has been compared with the structures of proteinase K from Tritirachium album Limber and Vibrio sp. PA44 in order to reveal structural explanations for differences in biophysical properties. The Serratia peptidase shares around 40 and 64% identity with the Tritirachium and Vibrio peptidases, respectively. The fold of the three enzymes is essentially identical, with minor exceptions in surface loops. One calcium binding site is found in the Serratia peptidase, in contrast to the Tritirachium and Vibrio peptidases which have two and three, respectively. A disulfide bridge close to the S2 site in the Serratia and Vibrio peptidases, an extensive hydrogen bond network in a tight loop close to the substrate binding site in the Serratia peptidase and different amino acid sequences in the S4 sites are expected to cause different substrate specificity in the three enzymes. The more negative surface potential of the Serratia peptidase, along with a disulfide bridge close to the S2 binding site of a substrate, is also expected to contribute to the overall lower binding affinity observed for the Serratia peptidase. Clear electron density for a tripeptide, probably a proteolysis product, was found in the S' sites of the substrate binding cleft.