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 titles:
Structure of a pathogenic mutant of human ornithine transcarbamoylase</td><td>Experiment number:
LS 1637</td></tr>
<tr><td>Beamline:
ID14 1 & 2</td><td>Date of experiment:
from: 10-May-00 to: 12-May-01
23-June-00 to: 24-June 00</td><td rowspan="2">Date of report:
27-Aug-01

Received at ESRF:</td></tr>
<tr><td>Shifts:
6+3</td><td>Local contact(s):
Drs Julien Lescar and Edward Mitchell</td></tr>
<tr><td colspan="3">Names and affiliations of applicants (* indicates experimentalists):
Vicente Rubio, Research Scientist and head proposer
Fernando Gil*. Ph.D. Student
Santiago Ramon-Maiques*. Ph.D. Student
Instituto de Biomedicina de Valencia CSIC
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Xavier Carpena* Ph.D. Student
Instituto de Biología Molecular de Barcelona-CSIC
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</table>

Report:

Although the time was allocated to project LS 1637, the Review Committees' comments clearly specified the following: *"Allocation of 6 shifts under project number LS 1637, to include projects LS1638 to LS1642."* These projects concerned the following topics:

- Aspartokinase from *Mycobacterium tuberculosis* (LS1638)
- Carbamoyl phosphate synthetase I from *Rana catesbeiana* (LS1639)
- N-Acetyl-L-glutamate kinase from *Escherichia coli* forming complex with aluminium fluoride (LS 1640)
- Carbamate kinase from *Enterococcus faecium* at high resolution (1641)
- *Pyrococcus furiosus* carbamoyl-phosphate synthetase trapped in the act of catalysis (LS1642).

Since there were technical problems in the initial visit that prevented full use of the shifts allocated, 3 additional shifts were allocated on a later date for beam ID14 2.

All but one of these projects concern enzymes that synthesize acylphosphate bonds using ATP as phosphoryl donor. The phosphoryl group acceptor is either a carboxylate group (N-acetyl-L-glutamate kinase and aspartokinase), carbamate (carbamate kinase and carbamoyl-phosphate synthetase) or bicarbonate (carbamoyl-phosphate synthetase I, which catalyzes two phosphorylation steps, one on bicarbonate and another on carbamate). The visits have allowed important progress to be made on our knowledge of three of these enzymes.

The *Pyrococcus furiosus* carbamoyl-phosphate synthetase has been characterised as a complex with ADP (Fig. 1A), and the nucleotide site has been delineated in detail (1). Despite hopes of having a crystalline "frozen" ternary complex including the other substrate, carbamoyl phosphate, no appropriate density for the latter has been observed, either in the presence of added ADP or in its absence. The enzyme structure of the pyrococcal enzyme not exposed to the nucleotide has resulted, nevertheless, in the observation of bound ADP, raising the

possibility that the nucleotide were sequestered by the enzyme. The structural data has opened the way to other biochemical studies in which the reasons for such sequestration are analyzed.

A dead-end ternary complex of the homologous enzyme, carbamate kinase, with ADP and the carbamate analog phosphonoacetate has been used to try to delineate the site, at a resolution of 1.5 Å, for the carbamate moiety in these enzymes. ADP has been found with full occupancy in essentially the same location and position than in *Pyrococcus furiosus* carbamoyl-phosphate synthetase, and a density that is not found in ligand-free carbamate kinase has also been observed and is highly suggestive of partial occupancy by phosphonoacetate of the carbamate site. The low occupancy possibly reflects the fact that phosphonoacetate is a poor competitive inhibitor with respect to carbamoyl phosphate with this enzyme (2). In this way, the entire substrate binding crevice has been nearly fully characterized.

The sites for the nucleotide and the acyl group substrate have been characterized to the highest degree in another homologous enzyme of the same family, N-acetyl-L-glutamate kinase from *Escherichia coli*. Crystalline complexes of this enzyme with ADP (a product), N-acetyl-L-glutamate (the phosphorylatable substrate) and AlF_4 (an analog of the migrating phosphoryl group in the transition state), have yielded detailed information at 2 Å resolution of the central ternary complex and has provided a structural framework (Fig. 1B) for proposing catalytic models for this enzyme family, which also includes aspartokinase, an enzyme for which the small crystal tested in the present experiments have not yielded yet relevant structural information (similarly, no structural information at atomic level has been obtained with crystals of frog liver carbamoyl phosphate synthetase I, another enzyme that, in addition to phosphorylating bicarbonate and carbamate, binds N-acetyl-L-glutamate as an allosteric activator; or with a clinical mutant of human ornithine transcarbamylase, an enzyme that also uses an acylphosphate, carbamoyl phosphate, although in this case it is the carbamoyl group rather than the phosphoryl, what is transferred to the acceptor group, which in this case is the ζ-amino group of ornithine).

Overall, our projects in ESRF have been highly successful in providing new light into a structurally poorly known enzyme family (EC 2.7.2.) and in defining the active centers and substrate binding in enzymes of this family. Tangentially, they have shed light into the reasons for the thermostability of *Pyrococcus furiosus* carbamoyl-phosphate synthetase.

1. Ramón-Maiques et al., *J. Mol. Biol.* 2000, 299:463-476
2. Marina et al., *Eur. J. Biochem.* 1998, 253:280-291

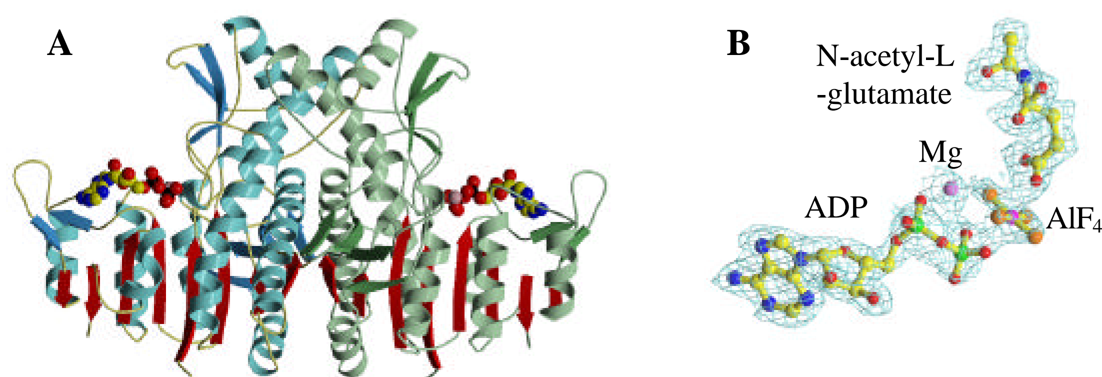

Figure 1. A) Ribbon representation of the CPS CK-like from *Pyrococcus furiosus* bound to MgADP (drawn in ball and stick). B) Structure representation of the transition state analog in the the reaction of N-acetyl-L-glutamate kinase. 2fo-fc map is contoured at 0.8 σ around the ligand molecules.