EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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:** Receptor-binding domain of T4 short fibre	**Experiment number**: 14-U-37
Beamline: ID14-4	**Date of experiment**: from: 2-3-2002 7am to: 3-3-2002 7am	**Date of report**: 27-4-2002
Shifts: 3	**Local contact(s)**: M. Walsh	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

***Ellen Thomassen**

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Report:

Introduction

Bacteriophage T4 is an elementary lifeform that displays a striking action: after recognising its bacterial host, it latches on and punctures the cell wall to inject its DNA, like a self-powered hypodermic syringe. T4 has an elongated head with icosahedral ends, containing its double-stranded DNA. For injection, it uses a contractile tail.

Adsorption of bacteriophage T4 to *Escherichia coli* is mediated by long and short tail fibres. Long fibres are responsible for initial, reversible, attachment, after which the short fibres extend and bind irreversibly to lipo-polysaccharide core and serve as inextensible stays during DNA-injection. Short tail fibres contain an N-terminal virus binding domain, a shaft and a C-terminal receptor-binding domain.

Previous work

Bacteriophage T4 short fibre protein (gene product 12) was co-expressed in *E. coli* with its chaperone gene product 57 (1). Folding experiments showed an unfolding transition at 56 degrees Celsius (2). Proteolysis of partially unfolded gp12 in the presence of EDTA identified a stable fragment of 33 kD (3) of which the structure was subsequently solved (4). This 33 kD fragment misses the N-terminal virus-binding domain and the C-terminal receptor-binding domain.

Later proteolysis experiments with gp12 partially unfolded in the presence of zinc ions identified a larger, 45 kD, fragment with an intact receptor-binding domain, which could also be crystallised. Tests of a crystal of the 45 kD fragment at ID14-4 showed, apart from extensive diffuse scatter, diffraction spots to around 1.5 Angstrom and a complete dataset was collected to 2.3 Angstrom resolution. However, we could not solve its structure by the molecular replacement technique.

Aims

We hoped the same mercury derivative used for the 33 kD fragment would also successful for structure solution of the 45 kD fragment. We aimed to collect one or more MAD datasets of the Hg-derivatised 45 kD fragment to be used for phasing.

Results

Four MAD datasets were collected around the Hg-edge. The data were used to obtain phases from which the structure was solved. The best electron density maps were obtained using only three of the high energy remote datasets in combination with the native dataset collected earlier on ID14-4.

The structure shows a new, rather peculiar, knotted fold and a central metal ion octahedrally coordinated by the NE2 atoms of six histidine side-chains (two from each monomer in the trimer). The structure also gives us clues as to where the receptor (the *E. coli* lipo-polysaccharide core) may bind. We are currently refining the structure, after which we will publish it giving due credit to the ESRF and the scientists involved.

References:

1. Burda MR, Miller S. Folding of coliphage T4 short tail fiber in vitro. Analysing the role of a bacteriophage-encoded chaperone. Eur J Biochem 1999 Oct;265(2):771-8
2. Burda MR, Hindennach I, Miller S. Stability of bacteriophage T4 short tail fiber. Biol Chem 2000 Mar;381(3):255-8
3. van Raaij MJ, Schoehn G, Jaquinod M, Ashman K, Burda MR, Miller S. Identification and crystallisation of a heat- and protease-stable fragment of the bacteriophage T4 short tail fibre. Biol Chem 2001 Jul;382(7):1049-55
4. van Raaij MJ, Schoehn G, Burda MR, Miller S. Crystal structure of a heat and protease-stable part of the bacteriophage T4 short tail fibre. J Mol Biol 2001 Dec 14;314(5):1137-46