

**Experiment title:**

Structure determination of the glutamate synthase from
Azospirillum brasilense

**Experiment
number:**

LS1517-LS1664

Beamline:

ID14-EH1
and BM14

Date of experiment:

20/11/1999 on ID14-EH1
from: 17/2/2000 to 19/2/00 on BM14.

Date of report:

29.02.00

Shifts:

1 shifts on
ID14EH1,
6 shifts on
BM14

Local contact(s):

H.Belrhali, W.Wakatsuki

Received at ESRF:

Names and affiliations of applicants (* indicates experimentalists):

(*)Claudia Binda and Andrea Mattevi

Dept. Genetics and Microbiology,

University of Pavia (Italy).

Report: Background: Glutamate synthase (GltS) participates in bacterial nitrogen assimilation metabolism. GltS is a tetramer of heterodimers, each dimer consisting of 2000 residues, three iron-sulphur clusters and a molecule of FMN and FAD. The catalytic cycle involves two-electron reduction of the FAD by NADPH, followed by electron transfer to the FMN through the iron-sulphur clusters. Next, FMN reduces the iminoacid formed by addition onto 2-oxoglutarate of the ammonia resulting from glutamine hydrolysis. Thus, GltS is able to employ the electron transfer from an "entry site" (the FAD) to an "exit site"

(the FMN) as driving force for the hydrolysis of Gln by an amidotransferase active centre. In this way, the directionality in the electron flow makes the enzyme catalysed reaction essentially irreversible.

Experiments carried out at the ESRF:

(1) A 3.0 Å native data set has been collected on ID14-EH1 with the following statistics: n° measurements=1059917, n° reflections=184349, $R_{\text{sym}}=10.2\%$ (41.2% in the highest resolution shell), completeness=98.7% (96.6%), $I/\sigma=8.2$ (3.1).

(2) A MAD experiment has been carried on BM14, on the Fe edge. The experiment has been finished two days ago (21st of February) and the data have not yet been fully processed.

Results: The native data set collected on ID14-EH3 extends to 3.0 Å resolution and is the best data set ever collected on glutamate synthase. It is self-evident that these data will be of critical importance for structure determination and refinement. We are not yet able to provide a full report on the MAD experiment carried out on BM14. However, two points can be already underlined:

-the crystal did not show any radiation damage, although it was exposed for four days at wavelengths around 1.7 Å which cause high crystal absorption (exposure time of 3 minutes, oscillation angle of 0.25°); -preliminary processing indicate that the data are of good quality and extend to 4.3 Å.

Taken together, these facts suggest that the experiment may allow phasing of the medium-low resolution reflections.