

Experiment title	Crystal structure of L-ala ligases implicated in cell wall synthesis
Experiment number	30-01-662
Dates of experiment	01 / 03 May 2004

The femABX protein family is constituted by enzymes involved in the synthesis of the peptidoglycan, the major component of the bacterial cell wall. These proteins catalyze the addition of an aminoacid on the petidoglycan precursor by using aminoacylated tRNA as a substrate. As this mode of action is unusual and has no equivalent in other steps of cell wall synthesis, we propose to develop antibiotics that will act on these novel targets. In the context of this project, we investigated the biochemical and structural studies of members of the FemABX family, particularly the FemX ligase of *Weissella viridescens*. Thanks to the beam time allocated on FIP in 2002 and 2003, we solved the structure at 1.7 Å resolution of the protein alone, a complex of the protein with its substrate, the UDP-MurNAc-pentapeptide, at 1.9 Å resolution (Biarrotte-Sorin et al, 2004), two mutant structures (K36M and Y254F) and the structure of the apo wild type in a second crystal form.

During the 6 shifts of the 30-01-662 experiment, we collected a SAD data set to 2.8 Å resolution of a related seleno-labelled enzyme in *Enterococcus faecium*, involved in antibiotics resistance. We are trying by now to solve this structure, but one third of the sequence is missing in the electronic density. Statistics of the data collection are summarized in Table I. We are currently tring to optimize the crystals in order to collect a higher quality data set.

Crystallographic Analysis	
Efaecium	
Data collection statistics	
Space group	P3 ₁ 21
Cell dimensions	a = b = 115.97 Å c = 68.27 Å
Wavelength (Å)	0.97972
Resolution (Å)	22.-2.60
Highest resolution shell (Å)	2.74-2.60
Number of observations	332,075
Number of unique reflections	16,468
R _{sym} (%)	13.5 (35.7)
I/σ(I)	3.7 (2.0)
Completeness (%)	99.3 (100.0)

Table I. Statistics of data collection. The values in parenthesis are for the highest resolution shell. Data have been processed with *MOSFLM*.

In parallel to this project, crystals of the β -lactamase OXA-13 at pH 8.5 in presence of bicarbonate, a class D β -lactamase, could be obtained in our laboratory. The aim of this project is to characterize the mechanism, with a special concern on the question of the carbamoylation of lysine 70, of the class D β -lactamases in order to gain structural and mechanistic insights to aid the design of new inhibitors of this enzyme family.

The protein crystallizes in the $P2_12_12_1$ space group with cell parameters $a = 45.81 \text{ \AA}$, $b = 113.20 \text{ \AA}$, $c = 125.97 \text{ \AA}$. During this experiment, a complete native data set (180 images, oscillation 1°) has been collected to 2.2 \AA resolution. The statistics of the data collection are summarized in Table I. The structure has been solved by molecular replacement using the OXA-13 at pH 4.5 solved in our laboratory (PDB id code 1H8Z). The structure is currently being refined.

Resolution (\AA)	35 -2.2
No. of observations	207 480
No. of unique reflections	33 353
R_{sym} (%)	6.2 (40.4)
Completeness (%)	92.4 (78.8)
$I / \sigma(I)$	27.1 (4.4)

Table I. Statistics of data collection. The values in parenthesis are for the highest resolution shell (1.8 - \AA). Data have been processed with *XDS*.

Publications

- S. Biarrotte-Sorin, A.P. Maillard, J. Delettré, W. Sougakoff, D. Blanot, K. Blondeau, J.-E. Hugonnet, C. Mayer & M. Arthur (2003). Crystallization and preliminary X-ray analysis of *Weissella viridescens* FemX UDP-MurNAc-pentapeptide:L-alanine ligase. *Acta Cryst.* **D59**, 1055-1057.
- S. Biarrotte-Sorin, A.P. Maillard, J. Delettré, W. Sougakoff, M. Arthur & C. Mayer. (2004) Crystal structure of *Weissella viridescens* FemX transferase and its complex with UDP-MurNAc-pentapeptide: Insights into FemABX family substrate recognition. *Structure*, 12, 57-67.
- A.P. Maillard, S. Biarrotte-Sorin, R. Villet, S. Mesnages, A. Bouhss, W. Sougakoff, C. Mayer & M. Arthur. Structure-Based Site-Directed Mutagenesis of the UDP-MurNAc-pentapeptide-binding Cavity of the FemX Alanyl Transferase from *Weissella viridescens*. *J. Bacteriology*. Submitted.