Experiment title	**Crystal structure of L-ala ligases implicated in cell wall synthesis**
Experiment number	**30-01-596**
Dates of experiment	**NO BEAM TIME ALLOCATED**

Experiment title	**Crystal structure of L-ala ligases implicated in cell wall synthesis**
Experiment number	**30-01-622**
Dates of experiment	**01 / 02 November 2003**

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, that involves 3 laboratories of the University of Paris VI and has been financed by the "Ministère de la Recherche" (ACI "Molécules et cibles thérapeutiques"), 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, we solved the structure at 1.7 Å resolution of the protein alone, and a complex of the protein with its substrate, the UDP-MurNAc-pentapeptide, at 1.9 Å resolution (Biarrotte-Sorin et al, 2004). In order to identify by a structural approach the specific substrate-enzyme interactions that are essential for the catalytic activity of the enzyme, we crystallized two FemX mutant, K36M and Y254F. In the same time, we obtained a second crystal form of the apo enzyme.

During these 6 shifts of the 30-01-622 experiment, data were collected for the two mutants and the second crystal form. The statistics of the data collection are summarized in Table I.

	K36M	Y254F	Crystal form II
Data collection statistics			
Space group	$P2_1$	$P2_1$	$P2_1$
Cell dimensions	a = 42.33 Å	a = 42.42 Å	a = 42.27 Å
	b = 101.30 Å	b = 101.33 Å	b = 101.54 Å
	c = 46.33 Å	c = 46.95 Å	c = 46.71 Å
	β = 103.02°	β = 102.40°	β = 103.19°
Wavelength (Å)	0.97957	0.97958	0.97953

Resolution (Å)	16.-1.95	16.-1.90	15.-2.00
Highest resolution shell (Å)	2.06-1.95	2.00-1.90	2.11-2.00
Number of observations	81,734	75,447	69,218
Number of unique reflections	27,741	28,895	25,437
R_{sym} (%)	6.6 (15.9)	7.7 (25.3)	8.5 (16.2)
I/σ(I)	6.6 (3.1)	7.1 (2.5)	4.8 (3.9)
Completeness (%)	94.5 (97.7)	94.9 (97.3)	98.2 (100.0)

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

The second crystal form was solved by molecular replacement, and the two mutant structures by difference Fourier synthesis and refined to 2.0, 1.9, and 1.95 Å resolution, respectively (Figure 1). A manuscript describing substrate binding site will be submitted in the journal *Journal of bacteriology*. Statistics of the refinement are summarized in Table II.

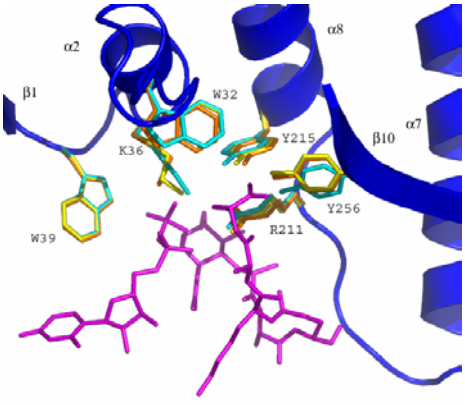

Figure 1. Close view of the superimposition of the $FemX_{Wv}$ UDP-MurNAc-pentapeptide binding cavity in the structures of the apo wild-type enzyme (yellow), UDP-MurNAc-pentapeptide enzyme complex (orange), and of the K36M mutant protein (cyan). The bound substrate and secondary structures of $FemX_{Wv}$ are colored in magenta and in dark blue, respectively.

	K36M	Y254F	Crystal form II
Resolution	16.-1.95	16.-1.90	15.-2.00
$R_{crystal}$ (%)	19.6	19.6	17.5
R_{free} (%)	23.3	23.3	21.4
Number of protein atoms	2687	2687	2688
Number of solvent atoms	367	355	487
Metal ions	1	1	0
Average B value-all ($Å^2$)	23.2	23.1	20.35
Average B value-all protein ($Å^2$)	21.8	21.8	18.06
Average B value-all solvent ($Å^2$)	31.4	33.0	33.03
r.m.s.d bond distance (Å)	0.006	0.006	0.005
r.m.s.d angle (°)	1.2	1.2	1.2
r.m.s.d dihedral (°)	23.1	23.1	23.1
r.m.s.d improper (°)	0.77	0.77	0.74

Table II. Statistics of refinement. The structures have been refined with *CNS*.

In parallel to this project, crystals of the β-lactamase OXA-13 at pH 8.5, a class D β-lactamase, could be obtained in our laboratory. The aim of this project is to characterize the mechanism 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 = 45.92 Å, b = 112.39 Å, c = 125.29 Å. During this experiment, a complete native data set (180 images, oscillation 1°) has been collected to 1.8 Å 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, and a manuscript is underway.

Resolution (Å)	28 -1.7
No. of observations	430 453
No. of unique reflections	66 519
R_{sym} (%)	3.8 (20.3)
Completeness (%)	92.5 (83.5)
I / σ (I)	28.8 (7.4)

Table I. Statistics of data collection. The values in parenthesis are for the highest resolution shell (1.8 - 1.7 Å). 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 Tranferase from *Weissella viridescens*. ***J. Bacteriology***. *Submitted.*