Experiment title	**Crystal structure of L-ala ligases implicated in cell wall synthesis**
Experiment number	**30-01-602**
Dates of experiment	**13 / 15 July 2002 - 13 / 14 December 2002**

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 (ref. 1). 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 two ligases in *Enterococcus faecalis*, BppA1 and BppA2 (ref. 2) and the homolog, FemX of *Weissella viridescens* (ref. 3).

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Crystals of a seleno-methionine derivative of *Weissella viridescens* FemX have been obtained and diffract to 2.1 Å resolution. During these 5 shifts of the 30-01-602 experiment, data were collected at the selenium peak and inflection wavelength. Because of the 16-bunch beam mode and weak diffracting crystals (3 minutes exposition was necessary), we could not collect diffraction data at remote wavelength. The statistics of the data collection are summarized in Table I.

	peak	inflection
Resolution (Å)	2.1	2.1
wavelength (Å)	0.98064	0.980973
sweep (°)	360	180
No. of observations	154237	77417
No. of unique reflections	20691	20758
R_{sym} (%)	4.4 (13.9)	5.7 (13.1)
Multiplicity	7.5 (7.5)	3.7 (3.8)
Completeness (%)	99.9 (99.9)	99.9 (99.9)
$I / \sigma (I)$	7.5 (6.2)	8.3 (4.8)

Table I. Statistics of data collection. The values in parenthesis are for the highest resolution shell (2.2 - 2.1 Å)

Thanks to the high data quality, the structure was finally solved by SAD method at the peak wavelength using the program *CNS*. Nine sites could be localized in the anomalous Patterson synthesis. Heavy atom refinement and phasing to 2.1 Å resulted in phases with a figure of merit of 0.406 and a phasing power of 1.70. After density modification, the map was submitted to the automatic wARP procedure. The resulting atomic model was completed using O (Jones & Kjeldgaard, 1997) and refined with *CNS*. The structure was then refined against the native data to 1.7 Å resolution (see report 30-01-602, date of experiment 13 June 2002 / 14 July 2002). Statistics of the refinement are summarized in Table II. Crystallization and structure determination of FemX have been published (Biarrotte-Sorin *et al*, 2003).

		Native	Complex [1]
$R_{crystal}$ (%)		17.6	17.2
R_{free} (%)		20.9	20.7
Number of proteins atoms		2688	2688
Number of substrate atoms		-	77
Number of solvent atoms		490	478
Metal ions		3	3
Average B value-all ($Å^2$)		18.2	16.3
Average B value-all protein ($Å^2$)		15.4	13.3
Average B value-all substrate ($Å^2$)		-	36
Average B value-all solvent ($Å^2$)		33.9	30.1
rmsd	bond distance (Å)	0.005	0.005
	angle (°)	1.3	1.3
	dihedrals (°)	23.2	22.8
	improper (°)	0.8	0.8

Table II. Statistics of refinement. [1] See below (date of experiment 13/15 december 2002).

Date of experiment	**13 December 2002 / 15 December 2002**

Isomorphous crystals of FemX in complex with the peptidoglycan precursor, the UDP-MurNAc-pentapeptide, have been obtained in condition similar to the apoenzyme crystals (Table III). A complete data set (210 ° sweep) has been collected. The statistics of the data collection are summarized in Table IV.

	Native	Complex
Space group	$P2_1$	$P2_1$
Cell dimensions	a = 42.03 Å	a = 42.201 Å
	b = 99.92 Å	b = 100.61 Å
	c = 45.84 Å	c = 46.538 Å
	β = 116.02 °	β = 116.64 °

Table III. Space group and cell parameters of apo and complexed FemX.

Wavelength (Å)	0.979711
Resolution (Å)	15.57 - 1.9
Highest resolution shell (Å)	2.0 – 1.9
Number of observations	102033
Number of unique reflections	27221
R_{sym} (%)	7.3 (19.0)
I/σ (I)	7.5 (3.7)
Completeness (%)	98.6 (98.0)

Table IV. Statistics of data collection. The values in parenthesis are for the highest resolution shell (2.0 - 1.9 Å)

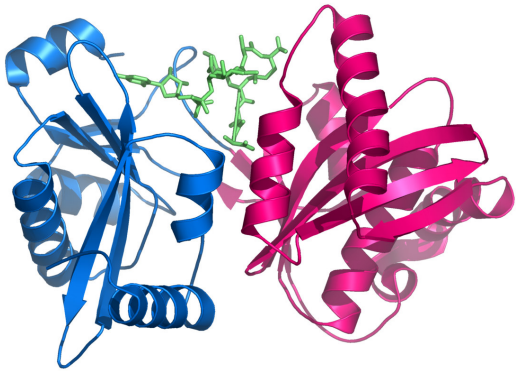

Figure 1. Structure of the FemX:UDP-MurNAc-pentapeptide complex.

The complex structure has been solved by difference Fourier synthesis and refined to 1.9 Å resolution (table II). A manuscript describing both the apo and the complexed protein is underway.

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. Manuscript in preparation.