

Experiment title	Crystal structure of L-ala ligases implicated in cell wall synthesis
Experiment number	30-01-602
Date of experiment	13 June 2002 / 14 June 2002

1. Crystal structure of L-ala ligases implicated in cell wall synthesis

We have recently identified two ligases in *Enterococcus faecalis*, BppA1 and BppA2 (ref. 1) involved in the synthesis of the peptidoglycane, the major component of the bacterial cell wall. These two ligases belong to a conserved family of proteins that include the products of the staphylococcal femABX and pneumococcal murMN. In the context of this project, we investigated first the biochemical and structural studies of one of the member of this family, the FemX protein of *Weissella viridescens*.

Crystallization assays for the FemX ligase resulted in reproducible needles of about 300 μ x 50 μ x 20 μ . The protein crystallizes in the P2₁ space group with cell parameters a = 42.03 Å, b = 99.92 Å, c = 45.84 Å and β = 116.02 °.

During the 3 shifts of the 30-01-602 experiment, a complete native data set has been collected to 1.7 Å resolution (oscillation range 1°, 30 sec exposition). The statistics of the data collection are summarized in Table I.

As no structure is available for this protein family, we crystallized a seleno-methionine derivative (see report 30-01-602, date of experiment 13 July 2002 / 15 July 2002).

Resolution (Å)	1.7
wavelength (Å)	0.979757
No. of observations	156408
No. of unique reflections	36921
R _{sym} (%)	4.4 (13.9)
Multiplicity	4.2 (3.7)
Completeness (%)	98.8 (97.7)
I / σ (I)	10.2 (3.7)

Table I. Statistics of data collection. The values in parenthesis are for the highest resolution shell (1.8 - 1.7 Å)

Ref 1. Bouhss A, Josseaume N, Allanic D, Crouvoisier M, Gutmann L, Mainardi JL, Mengin-Lecreulx D, van Heijenoort J, & Arthur M. (2001). Identification of the UDP-MurNac-pentapeptide:L-alanine ligase for synthesis of branched peptidoglycan precursors in *Enterococcus faecalis*. J. Bacteriol, **183**(17), 5122-5127.

2. Crystal structure of bovine GammaE

In parallel to this project, crystals of the bovine GammaE could be obtained in our Laboratory. Gamma-crystallin proteins corresponds to a multigenic family and are eye-lens

specific. The sequence of the seven members (Gamma A-F and GammaS) are highly conserved. But, despite a conserved 3D structure, they present very different chemical and physical properties. Till now, bovine GammaE could not be crystallized because of its pH specificity. X-ray diffusion studies by Tardieu and collaborators allowed to determine conditions that lead to high quality diffracting crystals.

The protein crystallizes in the P1 space group with cell parameters $a = 30.80 \text{ \AA}$, $b = 36.71 \text{ \AA}$, $c = 46.25 \text{ \AA}$, $\alpha = 100.06^\circ$, $\beta = 106.92^\circ$, $\gamma = 105.27^\circ$.

During the 3 shifts of the 30-01-602 experiment, a complete native data set has been collected to 1.65 \AA resolution. The statistics of the data collection are summarized in Table I. The structure has been solved by molecular replacement using the rat GammaE (PDB id code 1A5D). Refinement statistics are summarized in Table II. The coordinates of the refined structure have been deposited within the Protein Data Bank (PDB id code **1M8U**).

Resolution (\AA)	28 -1.65
No. of observations	45693
No. of unique reflections	20771
R_{sym} (%)	5.1 (10.4)
Multiplicity	2.2 (1.8)
Completeness (%)	92.4 (78.8)
$I / \sigma(I)$	8.1 (4.4)

Table I. Statistics of data collection. The values in parenthesis are for the highest resolution shell ($1.7 - 1.65 \text{ \AA}$)

Resolution (\AA)	20 - 1.65 \AA
No. of reflections	19708
Completeness (%)	91.1
R (%)	18.6
R_{free} (%)	21.6
No. of protein atoms	1487
No. of solvent	315

Table II. Refinement statistics.

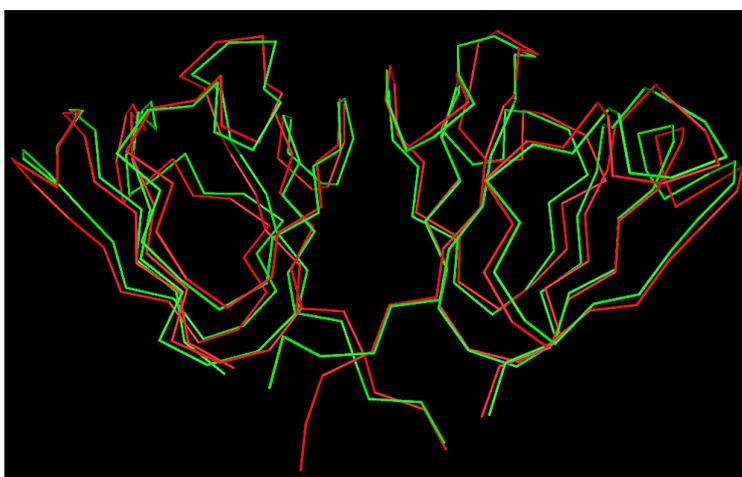


Figure I. $C\alpha$ trace of bovine GammaE (red) and rat GammaE (green) (rmsd after superposition 1.3 \AA).