

**Experiment title:**Outer-Membrane Phospholipase A from *E. coli***Experiment number:**

LS-690

**Beamline:**

D2AM

**Date of Experiment:**

from: 7-Feb. '97 7:00 to: 9-Feb. '97 7:00

**Date of Report:****Shifts:**

6

**Local contact(s):**

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**Report:**

Outer-Membrane Phospholipase A (OMPLA) is an enzyme located in the outer-membrane of Gram-negative *Escherichia coli* bacteria. This 31 kDa protein consists of 269 amino acid residues and is encoded by its structural gene *pldA*. The enzyme displays hydrolytic activity towards (phospho)lipids, shows broad substrate specificity and is strictly Ca<sup>2+</sup>-dependent. OMPLA shows no sequence homology with water soluble (phospho)lipases. However other *Enterobacteriaceae*, of which some are pathogenic such as *S. typhimurium* and *S. flexneri*, were reported to contain OMPLA encoding genes,

OMPLA is embedded in its own substrate, thus requires a strict regulation of its activity. In normally growing cells the enzyme seems to be dormant, only upon perturbation of the membrane OMPLA becomes active and starts hydrolysing phospholipids. The way of activation is not yet known but the active form is associated with a dimer, while monomeric OMPLA is hardly active or inactive. OMPLA is necessary for effective secretion of bacteriocins but it is unlikely that this is its primary function.

We have been able to grow crystals of this membrane protein, hexagonal rods are obtained in a vapour-diffusion hanging drop set-up. The initial reservoir solution contains 24-28% (v/v) MPD, 100 mM Bis-Tris buffer at pH 6.0, 1 mM CaCl<sub>2</sub> and 0-3% (v/v) PEG400. Initial phases have been determined by two three-wavelength MAD experiments on a seleno-methionine mutant and an iridium derivative. Phases were improved by solvent flattening and phase extension. This resulted in a well interpretable electron density map, in which a model has been built.

A native data set has been collected at D2AM beamline with statistics as presented in the table below. The resolution of data obtained at the D2AM beamline is 2.4 Å, substantially higher than the 2.7 Å obtained previously at the XI 1 beamline at the EMBL outstation in Hamburg. This data set is used for refinement, which is currently in progress.

Table. Data statistics of native OMPLA at D2AM beamline. Highest resolution shell: 2.44-2.40 Å.

Resolution		18-2.4 Å
No. refl.	observed	64321
	unique	14160
$\langle I/\sigma \rangle$	overall	18.9
	highest shell	7.6
Completeness	overall	84.0 %
	highest shell	51.0 %
$R_{\text{sym}}$	overall	4.6 %
	highest shell	14.2 %
$R_{\text{merge}}$		13.30 %