

**Experiment title:**Outer Membrane Phospholipase A,  
an integral membrane enzyme**Experiment  
number:**

LS 838

**Beamline:**

D2AM

**Date of Experiment:****from:** 7 Sep 1997      **to:** 8 Sep 1997**Date of Report:**

I 25 Feb 1998

**Shifts:**

3

**Local contact(s):**

M. Roth

*Received at ESRF :***04 MAR. 1998****Names and affiliations of applicants (\*indicates experimentalists):**

H.J. Snijder\*, A.oubrie\*, Prof. Dr. B-W. Dijkstra  
Lab. of Biophysical Chemistry  
University of Groningen  
9747 AG Groningen  
The Netherlands

**Report:**

Many important cellular processes take place in or at membranes. Yet very few structures of integral membrane enzymes are known. We study the outer membrane phospholipase A (OMPLA), an integral membrane enzyme widespread among Enterobacteriaceae, many of which are pathogenic. This enzyme hydrolyses membrane phospholipids. Although surrounded by its own substrate, it is normally dormant. Perturbations of the membrane trigger association of enzyme molecules into active dimers. OMPLA, together with the bacteriocin release protein, functions in secretion of bacteriocins.

We have solved the structure of this integral membrane enzyme to a resolution of 2.2 Å using synchrotron radiation (D2AM ESRF, X31/X11 EMBL-outstation DESY). The scaffold of the enzyme is formed by a 12 stranded anti-parallel  $\beta$ -barrel, with loops and turns connecting neighbouring strands. This architecture is strikingly similar to porins although OMPLA is considerably smaller. The active site is located at the outside of the barrel at the outer leaflet side of the membrane. The crystal structure represents the monomeric form of the enzyme. Focusing on the catalytic mechanism and dimerisation events, we have collected diffraction data of a crystal soaked with an inhibitor that forms a covalent complex with the active serine and that stabilises the dimer. Furthermore we have investigated this covalent inhibitor-enzyme complex by co-crystallisation. Small crystals of 0.1\*0.1\*0.01 mm<sup>3</sup> and different habit from native crystals have been obtained, however diffraction of these crystals proved to be very weak.

Data collection statistics for OMPLA soaked in hexadecylsulfonylfluoride. Value in brackets represents the highest resolution bin, 3.02-2.89 Å.

Spacegroup	P3 <sub>1</sub> 21			
Cell dimensions	a=b=77.496 Å	c=101.513 Å	$\alpha=\beta=90^\circ$	$\gamma=120^\circ$
Resolution	49-2.9 Å			
No. Reflections	observed	33950		
	unique	7697		
Rsym	9.32% (31.25%)			
<F>/<sigF>	23.2 (3.9)			