Structure and Regulation of an Integral Membrane Enzyme, Outer Membrane Phospholipase A

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Outer Membrane Phospholipase A (OMPLA) is an integral β-barrel membrane enzyme which can hydrolyse a broad range of phospholipids. It participates in secretion of colicins in *Escherichia coli*. In *Campilobacter* and *Helicobacter pylori* strains OMPLA is implicated in virulence. The activity of this enzyme is regulated by reversible dimerisation. Structural data on the factors that govern dimerisation of membrane proteins is scarce, however.

The monomers constituting the dimer (labelled A and B) interact with each other via the flat barrel surfaces. The dimeric complex is formed by association of monomeric units in a parallel fashion such that the active sites are located at the dimer interface at the outer leaflet side of the membrane. Approximately 1100 $Å^2$ of solvent accessible surface of each monomer is buried in the dimer-complex, representing 10.7% of total surface area. Interaction occurs almost exclusively in the membrane embedded parts, with a key interaction formed by hydrogen bond formation between glutamines 94 deeply embedded in the hydrophobic membrane environment.

From the known structures and the biochemical knowledge, we have constructued a model for the action and activation of OMPLA. Both calcium and substrate govern dimerisation. The main factor that controls dimerisation *in vivo* is most likely the substrate. Normally only the inner leaflet of the outer membrane contains phospholipids and OMPLA is present in the monomeric form. As activation concurs with the perturbation of the bacterial membrane, we propose that the relief of lipid asymmetry and presentation of the substrate induces activation by promoting dimerisation and calcium binding. The dimer-cofactor complex hydrolyses the substrate resulting in the formation of lyso-phospholipids and fatty acids which further destabilise the membrane bilayer presumably facilitating the release of colicins or virulence factors.

The dimeric form of OMPLA is obtained by covalent inhibition with hexadecane-sulfonyl fluoride (Figure 1). Since this inhibition-complex is unfit for product and substrate soaking experiments, we have searched for new crystallisation conditions. Besides the earlier reported crystallisation conditions [1, 2], OMPLA crystallises using a reservoir containing 0.1 M Ammonium Sulphate, 0.1 M Hepes buffer pH 7.6 and 18% PEG4000. These crystals were soaked in substrate and were flash-frozen. Data was collected at the BW7A beamline at the EMBL-outstation DESY-hamburg. Molecular replacement with the inhibited-dimer gave a clear solution for the rotation function and a more ambiguous solution for the translation function. Molecular replacement with the monomer as

search model gave similar solution, demonstration the dimeric nature of OMPLA in these new crystals.

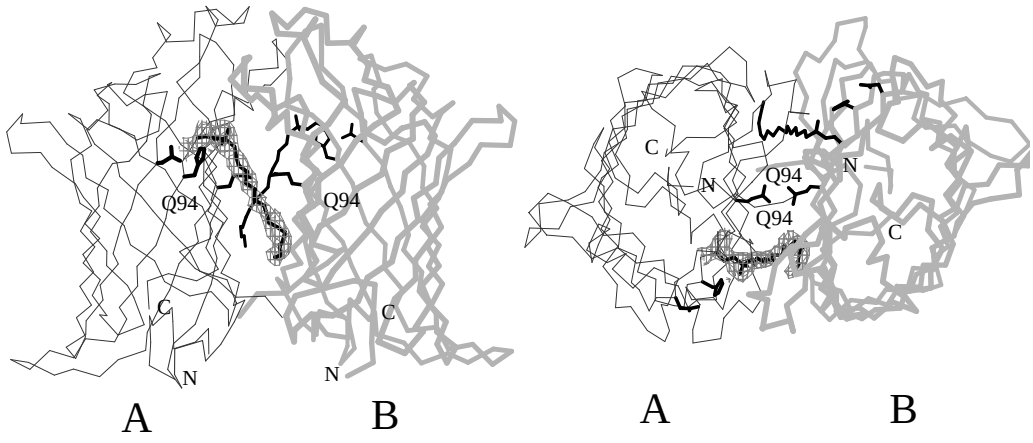

Figure 1: Dimeric inhibited OMPLA in two different orientations. Experimental electron density is shown for one of the inhibitors. Besides the active site residues, glutamines 94 are displayed in sticks. These residues are deeply embedded in the hydrophobic membrane and form polar interactions with each other.

References

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2. Snijder, H.J., *et al.* Structural evidence for dimerization-regulated activation of an integral phospholipase. *Nature* **401**, 717-721 (1999).