s8a.m4.o3 Structure and Regulation of Outer Membrane Phospholipase A. H.J. Snijder, J.H. van Eerde, R.L. Kingma, I. Ubarretxena, K.H. Kalk, H.M. Verheij, M.R. Egmond, N. Dekker and B.W. Dijkstra, *University of Groningen, Biophysical Chemistry, Protein Crystallography, Nijenborgh 4, 9747 AG Groningen, The Netherlands, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands.*
Keywords: membrane enzyme, calcium dependent phospholipase, dimerisation.

The gene encoding for outer membrane phospholipase A (OMPLA) is wide spread among Gram-negative bacteria, of which many are pathogenic. OMPLA is one of the few enzymes in the outer membrane of these bacteria. This 31 kDa protein hydrolyses phospholipids, displays broad substrate specificity, is strictly calcium dependent and shows no sequence homology with water soluble (phospho)lipases or with the structurally related porins. This phospholipase is surrounded by its own substrate but is dormant in normally growing cells. Perturbations of the membrane trigger the hydrolysis of phospholipids by forming active dimers[1].

The crystal structure of the monomer reveals a 12 stranded anti-parallel ß-barrel architecture. The active site is located at the end of a ß-strand and it harbors a unique catalytic triad comprising an asparagine, histidine and a serine.

Dimerisation results in formation of substrate binding pockets and functional oxyanion holes (that stabilizes the transition state). Monomer-monomer contacts are almost exclusively confined to the membrane embedded part of the protein and they comprise polar interactions in an otherwise hydrophobic environment[2].

All structural and biochemical evidence leads to an activation model of this membrane enzyme. Perturbation of the membrane results in phospholipid presentation to the outer leaflet. This triggers most likely dimerisation resulting in active complexes that hydrolyze the phospholipids. Production of lyso-phospholipid and fatty acids further destabilizes the outer membrane. This destabilization facilitates export of proteins, colicins or toxins out of the bacterium.

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s8a.m4.o4 The structure of the haem-copper oxidase from *Escherichia coli* and binding site for ubiquinone. J. Abramson[1], S. Riistama[2], G. Larsson[1], A. Jasaitis[2], M. Svensson-Ek[1], L. Laakkonen[2], A. Puustinen[2], S. Iwata[1] and M. Wikström[2]. [1]*Uppsala University, Department of Biochemistry, Biomedical Center Box 576, Uppsala S-75123, Sweden* [2]*Helsinki Bioenergetics Group, Department of Medical Chemistry, Institute of Biomedical Sciences and Biocentrum Helsinki, P.O. Box 8, FI-00014 University of Helsinki, Helsinki, Finland*
Keywords: membrane proteins, cytochrome bo_3 oxidase, quinon binding

Cell respiration is catalysed by the haem-copper oxidase superfamily of enzymes, comprising the cytochrome *c* and the quinol oxidases. These membrane proteins utilize different electron donors through dissimilar access mechanisms. We report the first X-ray structure for a quinol oxidase, cytochrome bo_3 from *Escherichia coli*. Single crystals of this integral membrane protein diffract X-rays to 3.5 Å resolution and belong to the orthorhombic space group $C222_1$. From the diffraction data, the cell dimensions were determined to be a=91.3 Å, b=370.3 Å and c=232.4 Å. These crystals have a solvent content of 59 percent and contain 2 molecules per asymetric unit. A search model generated from the structures of cytochrome *c* oxidase from *Paracoccus denitrificans* and the extrinsic domain of cytochrome bo_3 ubiquinol oxidase from *E. coli* was used for molecular replacement studies, resulting in a solution with sensible molecular packing. Contrary to current dogma, the membrane spanning region of subunit I in cytochrome bo_3 contains a cluster of charged conserved residues exposed to the interior of the lipid bilayer, which is revealed as a ubiquinone binding site. Comparative studies between this and other quinone binding sites unveil specific roles of these polar residues for electron and proton transfer in ubiquinol oxidase.