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OMPLA an OM enzyme

Marc Baaden
Christoph Meier
Mark S.P. Sansom
OMPLA: an outer membrane enzyme

- OMPLA
  - outer membrane lipase
  - dimerises to form active site
  - perturbed cell envelope required
- Ca$^{2+}$ ions stabilize dimer and active site
- Catalytic triad related to Ser proteases
Ompla dimer with calcium and bound inhibitor
OMPLA phospholytic activity

$A_{1/2}, \text{lyso-}A_{1/2}$ phospholipase mono/di-acyl lipase

- Escherichia coli
- 269 amino acids
- 20 a.a. signal seq.
- Cofactor calcium
- Broad specificity
- Widespread in Gram- bacteria

Apr. 2005, Slides
OmplA, JMB 2003, 331, 177
Marc Baaden, <baaden@smplinux.de>
Active site catalytic triad

Asn$_{156}$  His$_{142}$  Ser$_{144}$
OMPLA simulation systems
(Baaden, Meier & Sansom, JMB, 2003)

- XR structures by Snijder et al.
  (2.1 - 2.8 Å)

- Molecular Dynamics simulations

- In POPC bilayer

- 5 ns each (Cutoff) ... now 10 ns PME

- Monomer
  vs. dimer, Ca^{2+}
  vs. dimer-inhibitor complex, Ca^{2+}
Challenges in modeling OMPLA

Calcium

- parameters adapted from Shiratori & Nakagawa and Meulenhoff
- high affinity Ca^{2+} site (K_d=36\mu M)
- coordination controlled by distance restraints

- substrate parameterization based on Gromacs-87 forcefield

- membrane insertion

- symmetry
Calcium implementation

Table 2.3: The forcefield modification of the calcium ligands in dimeric OMPLA.

Modifications were made to the atomic charge and Lennard-Jones parameters (cf. appendix A) of the ligand atoms of calcium.

In the ab initio calculation the energy profile phospholipase A2 was generated and the nonbonded interaction energy parameters (for the Lennard-Jones and Coulomb interaction) were subsequently fitted to the calculated profile. The detailed derivation of these figures is discussed in appendix C. After a method by Shiratori et al.33 and Meulenhoff34.

Figure 2.7: Distance restraints in dimeric, calcium-containing OMPLA. An energetic penalty is added to the potential when the distance between specified pairs of atoms exceeds a threshold value. The potential form is quadratic below 1Å, and between 3Å and 4Å, and linear beyond 4Å. The cut-off distances are set such that the restraints do not act on distances close to those observed in the crystal structure of OMPLA. They are equivalent in strength to less than the weakest covalent bonds found in the protein (Force constant 200 kJ·mol⁻¹·nm⁻²).
Violation of Ca$^{2+}$ distance restraints

Simulation dim1

- Sum of violations
- Largest Violation
- Average Violation

Ca1-\(\gamma\)Ser$_{152}$

Ca2-\(\gamma\)Ser$_{152}$

Ca1-\(\gamma\)Ser$_{152}$

# Violations

Time / [ns]
Calcium coordination - $g(r)$

Calcium 1
- water
- monomer 1
- monomer 2

CN=8 ->

Calcium 2

Monomer 1

Monomer 2

water

$r(Ca1-O)$ distance /[nm]  r$(Ca2-O)$ distance /[nm]

0.2  0.3  0.2  0.3

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Structural drift (RMSD) from XR

![Graph showing structural drift over time for different regions: dim2, loops, all, and barrel.](image-url)
Dominant secondary structure

- **mono**
- **dim1**
- **dim2**
OMPLA RMSF vs B-factors
Active site dynamics

- catalytic triad fluctuates
- $\text{Ca}^{2+}$ stabilizes
- Substrate induces changes in monomer:monomer interactions

A

B

C

N156  H142  S144

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Stabilising role of calcium

Cα RMSF (Å)
Visual analysis vs. H-bond distances

N(H142) – HO(S144)

N156  H142  S144

O(N156) – HN(H142)

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Is there a fixed hydrogen bond network near Ca$^{2+}$?
Dominant water interactions

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Ca

84/100

0 ns  2.5 ns  5 ns
OMPLA dimer interface

- stacked aromatic rings
- complementary Leu bulges
- central polar Gln
- hydrogen bonds
HDS inhibitor binding pocket
Collapse of the substrate binding pocket

Contact surface

Substrate accessible surface

(surface: \[ \text{[Å}^2] \])

time: \([\text{ns}]\)

0 5
Interactions between monomers

dim 1

dim 2

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OmplA, JMB 2003, 331, 177

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Lipid-protein interaction

O(POPC) 39 %

25 %

NH(N156)
Many **biological nano-devices** are based on membranes (e.g. liposomes and nanosomes). To maintain membrane integrity under various conditions one might add specific proteins, like anti-freeze (glyco)proteins. In this context the Ompla enzyme could help to develop a security valve with respect to mechanical stress.

The Ompla enzyme functions as a kind of **security valve** in the bacterial outer membrane. It’s enzymatic cycle is activated by a mechanical deformation of the membrane, which triggers lysis of phospholipids. The mechanical trigger may be caused by an imbalance in membrane composition or by physical stress. Lysis and hence removal of phospholipids from the membrane helps to restore it’s integrity.

If the activity of Ompla could be controlled and fine-tuned, one might be able to develop self-regulating devices, with a capacity of dealing with a certain amount of environmental stress and membrane distorsion.

A first step is to fully uncover the enzymatic mechanism of Ompla, which is related to the presence of calcium ions, a specific hydration shell, dimerization and membrane distorsion.
Future directions

Further analysis
Lipid-protein interactions
Membrane
Water

Methodology
Extended simulations
PME vs Cutoff
micellar system

Enzymatic reaction
Mixed approaches (Car-Parrinello, QM/MM)
Additional documents

Movies and animations
http://www.baaden.ibpc.fr/pub/ompla/t_hdshow.avi
http://www.baaden.ibpc.fr/pub/ompla/t_omplarot.avi
http://www.baaden.ibpc.fr/pub/ompla/ompla_substrate_pocket.mov

Poster
http://glabaune.free.fr/omplpost.pdf

Further information at
http://www.baaden.ibpc.fr