The Interaction of Thionins with Model Pathogen Membranes

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Thionins

- Thionins are small (<5KDa) proteins produced as part of plants innate immune response system.

- All members are highly cationic (pI 9-11) which enables interactions with the anionic bacterial and fungal membranes.

- Most are also amphiphillic allowing partition into the hydrophobic core of the membrane.

- Thionins are cysteine rich, they contain between 8 and 10 cysteine residues forming 4-5 disulphide bonds per protein. Making these proteins structurally stable.
Antimicrobial Proteins which act at the membrane level.

- **Charge.**
- **Hydrophobic and hydrophillic domains (amphillicity).**
- **Hydrophobicity.**
- **Stable molecular conformation.**
- **Size.**
- The balance of these attributes modulates binding selectivity (prokaryotic/eukaryotic) and activity.
Fungal and bacterial membranes

Extracellular Space

- β(1,6)-D-glucan
- Chitin
- Fungal cell wall
- Phospholipid bilayer of the fungal cell
- β-(1,3)-glucan synthase
- Ergosterol

Fungal cytoplasm

Gram Negative

- Lipopolysaccharides
- Porins
- Outer membrane layer
- Peptidoglycan
- Periplasmic space
- Cell membrane
- Lipoproteins
- Membrane protein
- Membrane protein
Common Smudge (*Cochliobolus sativus*)

Glumbe Blotch (*Stagonospora nodorum*)

Tan spot (*Pyrenophora tritici-repentis*)

Stripe blight (*Pseudomonas syringie*)
α-purothioins

<table>
<thead>
<tr>
<th>α₁-Pth</th>
<th>KSCCRSTLGRNCYNLCRAARGAQKLCAGVRCRCKLSSGLSCPKGFPK</th>
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</thead>
<tbody>
<tr>
<td>α₂-Pth</td>
<td>KSCCRTTLGRNCYNLCRSSRGAKLCSSTVCRCKLTSGLSCPKGFPK</td>
</tr>
</tbody>
</table>

The image shows a comparison of two protein sequences, α₁-Pth and α₂-Pth, with highlighted amino acids that are different between the two.
α₁ and α₂-purothionin – same charge, differing hydrophobicity

Cation exchange

RP-HPLC

Elution volume / ml

μAu

Ammonium acetate conc. / mol

0 50 100 150 200 250 300 350

0 20 25 30 35 40

β-Pth α₁-Pth α₂-Pth
Aim
To determine the method by which thionins exert their seed defence activity, and determine how a difference in antimicrobial protein hydrophobicity effects protein membrane disruptive effects.
Comparing defense protein interactions with condense phase anionic phospholipid monolayers.
Brewster Angle Microscopy vs. time imaging

α1-Pth

22 mN m\(^{-1}\) before protein injection.

20 minutes after protein injection

90 minutes after protein injection

180 minutes after protein injection

α2-Pth

Surface Pressure / mN m\(^{-1}\)

Time / min
Equilibrium α1–Pth adsorbed DPPG monolayer
Equilibrium $\alpha_2$-Pth adsorbed DPPG monolayer
Monolayer results summary

Reflectivity

Bilayer models of the Gram negative bacterial outer membrane
Fabrication of asymmetric phospholipid:LPS membranes on silicon supports

Langmuir Blodgett + Langmuir Schaeffer =

Silicon + Si crystal

LC pump

Peek trough

Waste

J. R. Soc. Interface 2013, 10, 20130810.
DPPC : Rc-LPS bilayer

Reflectivity

Scattering Length Density

Q / Å⁻¹

Distance / Å
Changes Upon addition of α1-Pth

- blue line = before protein addition
- red line = after 0.01 mg / ml injected into cell
DPPC : Rc-LPS bilayer + α1-Pth

Reflectivity

Scattering Length Density / 10^-6 Å⁻²

Q / Å⁻¹

Distance / Å

Solution

Inner head group

Inner tails

Outer tails + embedded protein

Outer head group + adsorbed protein

SiO₂
Accurate Model of Bacterial Membranes for Structural Studies
Floating Model Gram Negative Bacterial Membranes: Fabrication

Langmuir Blodgett

Surface Pressure / mN m⁻¹

Monolayer area / cm²

Air

Water

Langmuir Schaeffer

Air

Water

“Outer Membrane”

“Inner Membrane”

Water Gap

DPPC

ThioPC

Rough LPS

Gold

Permalloy

Silicon
Antimicrobial protein interactions with the OM

Lactoferrin
pI 8.7
Floating Model Gram Negative Bacterial Membranes: Interaction Studies with Lactoferrin
Conclusions
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