Direct Reconstitution of OpaA into Lipid Membranes: A Membrane Protein Study

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Opa Proteins of Neisseriae

Host receptors involved in Opa-mediated phagocytosis

Hauck and Meyer, 2003

Opal
- 8-stranded β-barrel
- 27.1 kDa
- 238 amino acids
- pl: 9.6

Opa A
- 8-stranded β-barrel
- 26.7 kDa
- 236 amino acids
- pl: 9.9

β-sheets are 98.8% identical.
Cloning of OpaA Protein and Plasmid Prep
Transformation with OpaA pet 28b vector, N-His plasmid Into BL21 (DE3) E. Coli Cells

Expression and Purification
Purification via Co2+ Affinity Chromatography

Reconstitution Into Liposomes

Protein Preparation

Structure determination of Opa proteins and receptors
Heparansulfate binding studies and characterization
Verification of Opa protein structure and function
Lipids

**DMPC**
(14:0 PC) 1,2-dimyristoyl-sn-glycero-3-phosphocholine

**POPG**
(16:0-18:1 PG) 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (sodium salt)

**DMPE**
(14:0 PE) 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine

**DPPE**
(16:0 PE) 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine

**DMPS**
(14:0 PS) 1,2-ditetradecanoyl-sn-glycero-3-phospho-L-serine (sodium salt)

*Opal refolds in 100% DMPC!

**Vesicle Conditions**
- Lipid Type (head group, chain length, ionic strength)
- pH
- Temperature
Reconstitution

PS  S1   P1   S2   P2

Low-speed spin

High-speed spin

SDS-PAGE

Columbus Labs
University of Virginia
Optimal Refolding Trials

P2 = Pellet 2, Some folded protein
OpaA = Unfolded protein (25 kDa)

→ Folded protein migrates to ~23-24 kDa

<table>
<thead>
<tr>
<th></th>
<th>Lipid(s)</th>
<th>Protein Vol (uL), Conc in Lipid (μM)</th>
<th>pH</th>
<th>Estimated % Folded</th>
<th>Final Protein Conc. (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>90% DMPC, 10% POPG</td>
<td>5.73 uL, 4.7 uM</td>
<td>12</td>
<td>56%</td>
<td>0.14</td>
</tr>
<tr>
<td>2</td>
<td>60% DMPC, 40% POPG</td>
<td>51.0 uL, 4.7 uM</td>
<td>12</td>
<td>54%</td>
<td>0.25</td>
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<td>3</td>
<td>90% DMPC, 10% POPG</td>
<td>5.73 uL, 4.7 uM</td>
<td>12</td>
<td>52%</td>
<td>0.14</td>
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<tr>
<td>4</td>
<td>70% DMPC, 30% POPG</td>
<td>5.73 uL, 4.7 uM</td>
<td>12</td>
<td>51%</td>
<td>0.14</td>
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<td>5</td>
<td>85% DMPC, 15% POPG</td>
<td>5.73 uL, 4.7 uM</td>
<td>12</td>
<td>50%</td>
<td>0.14</td>
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<tr>
<td>6</td>
<td>50% DMPC, 50% DPPE</td>
<td>5.73 uL, 4.7 uM</td>
<td>11.7</td>
<td>50%</td>
<td>0.14</td>
</tr>
</tbody>
</table>

**Conditions:**
20mM Borate buffer,
Incubated at 37°C, 25 mg/mL conc. OpaA.
Folding Determinants

Hydrophobic Thickness

<table>
<thead>
<tr>
<th>Lipid</th>
<th>$C_{\text{chain}}$</th>
<th>$C_{\text{max}}$ (Å)</th>
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<tbody>
<tr>
<td>POPC</td>
<td>16:0/18:1</td>
<td>48</td>
</tr>
<tr>
<td>POPG</td>
<td>16:0/18:1</td>
<td>48</td>
</tr>
<tr>
<td>DMPC</td>
<td>14:0</td>
<td>36</td>
</tr>
<tr>
<td>DOPC</td>
<td>18:1</td>
<td>48</td>
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</tbody>
</table>

Tanford, *The Hydrophobic Effect*, 1973

OmpA (a β-barrel protein) in a lipid bilayer.

Opal: 10 residue/ transmembrane segments, ~3.
4 Å/residue
Approximate hydrophobic thickness: 34Å
Folding Determinants

Electrostatics

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### Table: Approximate charge of extracellular loops at pH 12

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<thead>
<tr>
<th></th>
<th>HV1</th>
<th></th>
<th>Net</th>
<th></th>
<th>Net</th>
<th></th>
<th>Net</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Opal</td>
<td>+2</td>
<td>-3</td>
<td>-1</td>
<td>1.5</td>
<td>-4</td>
<td>-2.5</td>
<td>0</td>
<td>+3.5</td>
</tr>
<tr>
<td>OpaA</td>
<td>+1</td>
<td>-1</td>
<td>0</td>
<td>+2.5</td>
<td>-3</td>
<td>-0.5</td>
<td>0</td>
<td>+3.5</td>
</tr>
</tbody>
</table>

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Conclusions & Future Directions

• OpaA has been expressed and purified.

• Liposome reconstitution of OpaA with a yield of greater than 50% refolded protein.

• Further studies of hypervariable loop and sequence variation for refolding optimization.

• OpaA-HSPG and OpaA-heparin binding interactions and structures will be characterized using fluorescence, as well as electron paramagnetic resonance (EPR) coupled with site-directed spin labeling.
Support for this research provided by the NIH (RO1 GM087828-02), NSF (CAREER award MCB0845668), the Jeffress Memorial Trust, and the Charles Henry Leach, II Foundation.