Conformational Changes of Gai1 nucleotide exchange catalyzed by Ric-8A

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Conformational Changes of Gαi1 Nucleotide Exchange Catalyzed by Ric-8A

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Introduction

- G-Proteins regulate several cellular processes, and when defective, have been associated with several endocrinial disorders.
- Heterotrimeric G-Proteins are regulated by the binding of GDP (inactive state) and GTP (active state) on the α subunit.
- Ric-8A is a ~60-kDa cytosolic protein that acts as a GEF to exchange GDP for GTP as shown in schematic 1 in vitro, and a chaperone for the Gα subunit in vivo.
- Previous studies with DEER spectroscopy have demonstrated a Ric-8A induced structural change in which, the Ras domain pivots away from the helical domain, exposing the nucleotide binding site and resulting in the release in GDP.
- The aim of this study is to elucidate the kinetics of Ric8A induced conformational changes in Gαi1 and potentially, any uncharacterized intermediates such as Gαi1-GDP:Ric-8A, using stopped-flow FRET ( Förster Resonance Energy Transfer) spectroscopy.
- FRET is a distance dependent physical process where energy is transferred from an excited fluorophore (donor) to another fluorophore (acceptor) by intramolecular long-range dipole-dipole coupling.
- To analyze Ric8A induced Gαi1 conformational changes, cysteines introduced in the Ras-like and Helical domain (figure 3), from a Gαi1 construct with cysteines removed (figure 2), will be labeled with acceptor and donor fluorescent dyes.
- Stopped flow enables the measurement of FRET upon the addition of Ric-8A and allows the FRET signal to be monitored as Gαi1 undergoes conformational changes.

**Purification of Hexa I-Gαi1**

<table>
<thead>
<tr>
<th>E-Coli Protein Expression</th>
<th>BL21 (DE3) - RILS</th>
<th>P-DEST 15 Vector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Culture</td>
<td>TB Media</td>
<td>200 RPM, 37°C, OD 0.6</td>
</tr>
<tr>
<td>Affinity column</td>
<td>GST-Beads</td>
<td>Separate Gαi1 from Supernatant</td>
</tr>
<tr>
<td>Ion Exchange</td>
<td>Q-Column</td>
<td>Separate Gαi1 based on PI</td>
</tr>
<tr>
<td>Size-Exclusion</td>
<td>Superdex-200</td>
<td>Separate based on size pure Gαi1</td>
</tr>
</tbody>
</table>

**RIC-8A GEF Mechanism**

GDP + R → GDP:Ric → GTP with FRET

G = Gαi1
R = Ric-8A
D = GDP
T = GTP

Schematic 1. Ric-8A acts as a GEF in vitro to mediate the release of GDP from GDP:Gαi1 for GTP, resulting in an active conformation of Gα. This mechanism is poorly understood, and the kinetics have not been well established.

**Hexa I-Gαi1 Constructs**

Green: Helical domain
Gray: Ras-like domain
Red: GDP

**Size-Exclusion Hexa I-Gαi1**

**Potential Stopped-Flow Results**

Gαi1 → GDP ⇌ GDP:Ric-8A ⇌ Gαi1 · Ric-8A

**Acknowledgments**

Dr. Sprang’s Lab: Stephen Sprang, Levi McClelland, Cindyé Yates-Hansen. NIH RO1 GM 105993

**Future Directions**

- Label Hexa I-Gαi1 with fluorescent dyes, and run stopped flow to determine the kinetics of Ric-8A induced conformational changes of Gα.