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

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

Introduction
• G-Proteins regulate several cellular processes, and when defective, have been associated with several endocrinological 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>Purification</th>
<th>E-Coli Protein Expression</th>
<th>Cell culture</th>
<th>Affinity column</th>
<th>Ion Exchange</th>
<th>Size-Exclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL21 (DE3) - RipL</td>
<td>P-DEST 15 Vector</td>
<td>TB Media</td>
<td>GST-Beads</td>
<td>Q-Column</td>
<td>Superdex 200</td>
</tr>
</tbody>
</table>

Figure 2. 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
Grey = Ras-like domain
Red = GDP

Figure 3. SDS-PAGE during purification of Hexa I-Gαi1 utilizing a GST column. Lane 1 displays the ladder, Lane 2 lysate, Lane 3 flow-through, Lane 4 wash, Lane 5 GST beads, Lane 7 elution post TEV-digestion.

Figure 3. Yellow spheres represent cysteines that were introduced for labeling. Three FRET pairs containing either 90-238, 90-209, and 90-214 will be used for this study. Obtained from protein data bank (1G1T).

Acknowledgments

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