**X-ray Crystallographic Studies of Human Rheb (P71A)-GppNHp**

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**Introduction**

The mammalian TOR (mTOR) pathway is a key regulator of cell growth and proliferation and increasing evidence suggests that its deregulation is associated with human diseases, including cancer and diabetes. The mTOR pathway integrates signals from nutrients, energy status and growth factors to regulate many processes, including autophagy, ribosome biogenesis and metabolism. Ras homolog enriched in brain (Rheb) represents a novel and unique subfamily of the Ras superfamily GTP-binding proteins. Rheb displays unique biological and biochemical properties different from other small GTPases and functions as an important mediator between the tumor suppressor proteins TSC1 and TSC2 and the mammalian target of rapamycin to stimulate cell growth. Comparison of hRheb with Ras reveals substantial conformational difference in the switch II region, so we mutated a series of residues in Rheb switch II, and carried out structural and functional studies of these mutants. This work would give an explanation for the unique biochemical characterization of Rheb and provide a molecular mechanism of regulation between them. Meanwhile, it may also be helpful for the study of the relationship between Rheb and other small G-proteins and guide the design of treatments for tuberous sclerosis and cancers.

**Method and Results**

Expression and purification of Rheb mutants were carried out as described previously [1]. Sparse-matrix crystallization screening with the Crystal Screen I and Crystal Screen II kits (Hampton Research) was performed using the hanging-drop vapor diffusion method at 4°C. Crystal of RhebP71A-GppNHp was mounted on a cryoloop and flash-frozen in liquid nitrogen. Data collection was carried out using the ADSC CCD detector of BL6A or 5A at PF. Data processing and scaling were performed using the HKL2000 suite.

**Table 1. Statistics of X-ray diffraction data**

<table>
<thead>
<tr>
<th>Resolution(A)</th>
<th>60.00-2.70 (2.90-2.70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Space group</td>
<td>C2</td>
</tr>
<tr>
<td>Cell parameters(A)</td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>119.3</td>
</tr>
<tr>
<td>b</td>
<td>90.7</td>
</tr>
<tr>
<td>c</td>
<td>95.8</td>
</tr>
<tr>
<td>(\beta)</td>
<td>121.6</td>
</tr>
<tr>
<td>(\gamma)</td>
<td>90.0</td>
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<tr>
<td>Observed reflections</td>
<td>32,875</td>
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<tr>
<td>Unique reflections(I&gt;2\sigma(I))</td>
<td>5,037</td>
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<tr>
<td>Completeness(%)</td>
<td>98.2(99.1)</td>
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<tr>
<td>Rmerge</td>
<td>0.047(0.109)</td>
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<tr>
<td>l(cil)</td>
<td>25.4(7.2)</td>
</tr>
</tbody>
</table>

**Fig. 1. Overall structure of RhebP71A-GppNHp**

**References**


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