Rab25- Ras-related GTP-binding protein 25 with bound GDP

PDB:2OIL

Revision

Revision Type:created
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Entry Clone Accession:NM_020387
Entry Clone Source: Codon Devices
SGC Clone Accession:
Tag:N-terminal hexahistidine tag
Host:E.coli. BL21 (DE3) codon(+) RIL

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsEDYNFVFKVVLIGESGVGKTNLLSRFTRNEFSHDSRTTIGVEFSTRTVMLGTAAVKAQIWDTAGLERY
RAITSAYYRGAVGALLVFDLTKHQTYAVVERWLKELYDHAEATIVVMLVGNKSDLSQAREVPTEEARMFAENNGLLFLETSALDSTN
VELAFETVLKEIFAKVSKQ

Vector:p28a-thrombin-lic

Growth

Medium:
Antibiotics:
Procedure: The target protein was expressed in E.coli. BL21 (DE3) coden (+) RIL containing the plasmid. The 100 mL overnight culture in Luria-Bertani medium was inoculated into 1.8 L of Terrific Broth medium in the presence of 50 µg/mL of kanamycin and 50 µg/mL chloramphenicol at 37 ºC and grown to an OD600 ~ 3.0 in the SGC LEX bubbling system. The culture was induced by isopropyl-1-thio-D-galactopyranoside at the final concentration of 1.0 mM and grown overnight at 18 ºC. The culture was harvested with centrifugation. Pellets were flash frozen and stored at -80 ºC.

Purification

Procedure
Column 1: Ni-NTA beads
Procedure: 1 ml of Ni-NTA suspension solution was added into 100 ml cell lysis supernatant solution. The mixture was shaken for 1 hour at 4 ºC. Beads were collected by centrifugation at 2500 rpm for 5 minutes. Beads were washed with 75 ml washing buffer, then collected by

centrifugation. Protein was eluted with 15 ml elution buffer. Thrombin (1 unit/mg protein) was added into the eluted protein solution, and the solution was shaken at 4 ºC overnight to remove the His tag.

Column 2: Size exclusion chromatography (Superdex 75 26/60)
The fractions eluted off the Ni-affinity column were applied to a Superdex 75 column equilibrated in SEC buffer at a flow rate of 2.0 ml/min. Eluted fractions were 95% pure as judged by SDS-PAGE.

Protein concentration: Amicon ultra centrifugal filter with a 5 kDa cut off in SEC-buffer

Extraction

Procedure
Cells were thawed and re-suspended in 100 mL binding buffer (10 mM Tris pH 7.5, 0.5 M NaCl, 5 mM imidazole) with 0.5% CHAPS (Sigma) and 1 mM phenylmethyl sulfonyl fluoride (PMSF), 0.5% (v/v) protease inhibitor cocktail (Sigma), 1 mM Benzamidine, 1600 units Benzonase (Sigma), and lysed with a microfluidizer. The lysate was centrifuged at 16000 rpm for 45 min and the supernatant was used for subsequent steps of purification. All the extraction steps were carried out at 4 ºC.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were obtained using the vapor diffusion method. The protein solution (27 mg/ml) contained 5 fold molar of GDP and $MgCl_2$. 0.5 µl of the concentrated protein was mixed with 0.5 µl of a well solution containing 20% PEG3350, 0.2 M NH4Cl. Crystals appeared after two days at 18°C.
NMR Spectroscopy:
Data Collection: Crystals were cryo-protected using a mixture of PEG3350 and PEG400, and flash frozen in liquid nitrogen. Diffraction data were collected at Rigaku FR-E with Rigaku R-AXIS IV++ detector to 2.3 Å.
Data Processing: