GIMAP1

PDB:3LXW

Revision

Revision Type:created
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Entry Clone Accession:AT72-F5:BC040736.1
Entry Clone Source:MGCHPC09G-H01
SGC Clone Accession:mhhhhhhssgrenlyfq*g
Tag:GIMAP1:E25-R253
Tag not removed
Host:BL21-V2R-pRARE2

Construct

Prelude:
Sequence:

mhhhhhhssgrenlyfqgESTRRLILVGRTGAGKSATGNSILGQRRFFSRLGATSVTRACTTGSRRWDKCHVEVVDTPDIFSSQVSK
TDPGCEERGHCYLLSAPGPHALLLVTQLGRFTAQDQQAVRQVRDMFGEDVLKWMVIVFTRKEDLAGGSLHDYVSNTENRALRELVAE
CGGRVCAFDNRATGREQEAQVVQLLGMVEGLVLEHKGAHYSNEVYELAQVLRWAGPEERLRRVAERVAARVQR

Vector:pET28-MHL

Growth

Medium: Terrific Broth medium in the presence of 50 mg/mL kanamycin and 25 mg/mL chloramphenicol
Antibiotics:
Procedure:LEX Bubbling. The target protein was expressed in *E. coli* by inoculating 100 mL of overnight culture grown in Luria-Bertani medium into a 2 L of growth medium at 37 °C. When OD_{600} reached ~3.0, the temperature of the medium was lowered to 15 °C and the culture was induced with 1 mM IPTG. The cells were allowed to grow overnight before harvested and flash frozen in liquid nitrogen and stored at -80 °C.

Purification

Procedure
The lysate was centrifuged at 15,000 rpm for 45 minutes and the supernatant were mixed with 5 mL 50% flurry of Talon Cobalt beads and incubated at 4 degrees Celsius on rotary shaker for one hour. The mixture was then centrifuged at 2300 rpm for 5 min and the supernatant discarded. The beads were then washed with washing buffer containing 30 mM and 75 mM Imidazole, and finally the elution buffer. The flow-through was collected and treat with TEV protease for two days. The protease and uncut protein were removed by flowing the solution through a Talon cobalt open column and the flow-through were further purified by a Superdex-75 gel filtration column pre-equilibrated with gel filtration buffer. Fractions containing the protein were collected and added 5mM $MgCl_2$ and concentrated with Amicon Ultra-15 centrifugal filter. GDP was then added to the concentrated protein solution to a final concentration of 5 mM. The purity of the preparation is tested by SDS-PAGE to be greater than 95%.

Extraction

Procedure
Frozen cells from 2L TB culture were thawed and resuspended in 150 mL extraction buffer with freshly added 0.5% CHAPS, and supplemented with protease inhibitor cocktail (SIGMA Catalog # P8849), and 3 uL benzonase (Sigma Catalog # E1014, 250U/uL), and lysed using microfluidizer at 15,000 PSI.
Concentration:29.9 mg/mL
Ligand
GDP, Mg^{2+}**MassSpec:**Native protein expected: 27705.22 (uncut), 25568.9
Measured: 27688.1 (uncut), 25552.0
A delta of -17 Da prompts a mutation from Asn to Pro but this needs to be confirmed by DNA sequencing.
Crystallization: Crystal used for structure determination was grown in Red Wings screen condition E10 from initial screen.
Crystal was grown in 25% W/V PEG3350, 0.2 M NaCl, 0.1 M HEPES pH 7.5, in the presence of 1:100 (w/w) dispase and 5mM GDP in sitting drop setup.
Crystals grow to a mountable size within 3 days.
Cryoprotectant used Paratone-N
NMR Spectroscopy:
Data Collection:
Data Processing: