Rab1A

PDB:2FOL

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source:MGC
SGC Clone Accession:
Tag: His-tag with integrated thrombin protease site before the last Ser:
MGSSHHHHHHSSGLVPRGS
Host:E. coliBL21-CodonPlus (DE-3)-RIL

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsPEYDYLFKLLLIGDSGVGKSCLLLRFADDTYTESYISTIGVDFKIRTIELDGKTIKLQIWDTAGQERF
RTITSSYYRGAHGIIVVYDVTDQESFNNVKQWLQEIDRYASENVNKLLVGNKCDLTTKKVVDYTTAKEFADSLGIPFLETSAKNATN
VEQSFMTMAAEIKKRMG

Vector:p28a-LIC

Growth

Medium:
Antibiotics:
Procedure: We prepared the seeds by inoculating glycerol stock of E. coli cells BL21-CodonPlus (DE-3)-RIL into 100 mL of Luria-Bertani medium. After overnight growth, all of the seeds were inoculated into 1.8 L of Terrific Broth medium in the presence of 50 µg/mL of kanamycin and 50 µg/mL chloramphenicol at 37ºC and grown to an OD600 between 3-5. Cells were then induced by isopropyl-1-thio-D-galactopyranoside at the final concentration of 1.0 mM and grown overnight at 18ºC in the SGC LEX bubbling system.

Purification

Procedure
The supernatant was passed through DE52 (Whatman) column equilibrated with the binding buffer, and then loaded onto 5 ml HisTrap HP (Amersham) equilibrated with the same binding buffer at 4ºC. The HisTrap HP column was steply washed with 25 ml of binding buffer, 25 ml of binding buffer with 30 mM imidazole, and 25 ml of binding buffer with 50 mM imidazole. The His-tagged protein was eluted by linear gradient of imidazole from 50 mM to 500 mM in 50 ml.

The eluted protein peak fractions detected by UV280 nm were combined and further purified by gel filtration column superdex 75 with a buffer containing 20 mM HEPES pH 8.0, 500m M NaCl, 1 mM DTT. Protein peak fractions were combined, GDP (Sigma) 5 times of the Rab1A in molarity, and $MgCl_2$ to the final concentration of 5 mM MgCl2 were added before concentration.

The protein was concentrated using an Amicon Ultra centrifugal filter to the final volume of 0.5 mL. The protein concentration estimated by Bradford to be 112.5 mg/mL. About 54.2 mg of protein was obtained from 1.8 L of cell culture.

Extraction

Procedure
Cultures were centrifuged and the cell pellets were harvested and stored at -80 ºC before use. Cells were thawed and suspended in 100 mL the 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) and lysed with microfluidizer. The lysate was centrifuged at 15000 rpm for 30 min and the supernatant was used for subsequent steps of purification. All the extraction steps were carried out at 4ºC.
Concentration:112.5 mg/mL
Ligand
GDP**MassSpec:**
Crystallization: Purified RAB1A was crystallized using the sitting drop vapor diffusion method at room temperature. Crystals grew in 50 days when the protein (112.5 mg/ml) was mixed with the reservoir solution in a 1:1 volume ratio, and the drop was equilibrated against a reservoir solution containing 25% PEG3350, 0.2M MgCl2, 0.1M Tris, PH8.5. The crystals were flash frozen with the mother liquor.
NMR Spectroscopy:
Data Collection:
Data Processing: