Rab26

PDB:2G6B

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:46361978
Entry Clone Source:MGC
SGC Clone Accession:
Tag:N-terminal: His-tag integrated at the N-terminal of the protein was cleaved by thrombin protease, resulted in the addition of two residues (GS) attached to the first amino acid of the encoded protein
Host:E. coli BL21-CodonPlus (DE-3)-RIL

Construct

Prelude:
Sequence:

gsGVDFYDVAFKVMLVGDSGVGKTCLLVRFKDGAFLAGTFISTVGIDFRNKVLDVDGVKVKLQMWDTAGQERFRSVTHAYYRDAHAL
LLLYDVTNKASFDNIQAWLTEIHEYAQHDVALMLLGNKVDSAHERVVKREDGEKLAKEYGLPFMETSAKTGLNVDLAFTAIAKELKR
RSMKAP

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 4degC. The HisTrap HP column was washed with 25 ml binding buffer and then 25 ml binding buffer with 50 mM imidazole, and 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 Thrombin protease was added to the eluted protein and the protease digestion reaction was carried out at 4degC overnight. After 16 hours thrombin digestion, almost all protein was His-tag removed, as illustrated by SDS-PAGE. The untagged protein was separated from the tagged protein by passing through Ni-NTA resin and further purified by gel filtration column superdex 75. The Gel filtration buffer contains 20 mM HEPES pH6.7, 500m M NaCl, 1 mM DTT. Protein peak fractions were combined and concentrated using an Amicon Ultra centrifugal filter to the final volume of 0.75 mL. The protein concentration estimated by Bradford to be 14.1 mg/mL. About 10.6 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 degC 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:
Ligand
guanosine-5Â□-(beta, gamma)-imidotriphosphate (GppNHp)**MassSpec:**
Crystallization: Purified Rab26 was crystallized using the sitting drop vapor diffusion method at room temperature. Crystals grew in two days when the protein (14.1 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, 0.1M NH4SO4, 0.1M HEPES, pH7.5. The crystals were flash frozen with the mixture of paratone-N: mineral oil at 1:1 as the cryo solution.
NMR Spectroscopy:
Data Collection:
Data Processing: