RALGDS

PDB:3KH0

Revision

Revision Type:created
Revised by:created
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Entry Clone Accession:MGC:AT65-H11, BC059362.1
Entry Clone Source:MGC
SGC Clone Accession:HPC09Q-F03
Tag:N-terminal tag: mhhhhhhssgrenlyfq*g
Host:BL21-V2R-pRARE2

Construct

Prelude:RALGDS:N793-F914
Tag was not removed.
Sequence:

mhhhhhhssgrenlyfqgNQQVGDCCIIRVSLDVDNGNMYKSILVTSQDKAPAVIRKAMDKHNLEEEEPEDYELLQILSDDRKLKIP
ENANVFYAMNSTANYDFVLKKRTFTKGVKVKHGASSTLPRMKQKGLKIAKGIF

Vector:pET28-mhl (GI:134105571)

Growth

Medium:
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 Terrific Broth medium in the presence of 50 µg/mL kanamycin and 25 µg/mL chloramphenicol at 37 degC. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 °C and the culutre was induced with 0.5 mM IPTG. The cells were allowed to grow overnight before harvested and flash frozen in liquid nitrogen and stored at -80 degC.

Purification

Procedure
The lysate was centrifuged at 16,000 rpm for 1 hour and the supernatants were mixed with 12 mL 50% flurry of Ni-NTA beads and incubated at 4 degC on rotary shaker for 0.7 hour. The mixture was then centrifuged at 2300 rpm for 5 min and the supernant discarded. The beads were then washed with washing buffers containing 5 mM and 10 mM Imidazole, and finally the bound protein was eluted with the elution buffer. The flow-trough was collected and further purified by a Superdex-75 gel filtration column pre-equilibrated with gel filtration buffer. Fractions

containing the protein were collected and concentrated with Amicon Ultra-15 centrifugal filter (cut-off 5 kDa). The purity of the preparation is tested by SDS-PAGE to be greater than 95%.

The His6-tag was not removed for crystallization.

Extraction

Procedure
Frozen cells from 4L TB culture were thawed and resuspended in 500 mL Binding buffer with freshly added 0.5% CHAPS, and supplemented with 1.7 mL protease inhibitor cocktail (SIGMA Catalog # P8849), and 10 microL benzonase (Sigma Catalog # E1014, 250U/microL), and lysed using sonication at 120W for 8 minutes.
Concentration:23.0 mg/mL
Ligand
MassSpec: Native expected 15998.3 Da, measured 15999.6 Da.
Crystallization: Crystallization was setup using in situ proteolysis method in sitting drops with Red Wings and SGC-I screens. Diffracting crystals were found from initial screen drops.

Crystal used for structure determination was grown in 2.0 M (NH4)2SO4, 2.0% PEG400, 0.1M Na HEPES buffer pH 7.5, with 1:100 Endoproteinase Glu-C (w/w) in sitting drop setup. 0.3 uL protein solution plus 0.3 uL well solution was used. Crystals grow to a mountable size in 3 days. Paratone was used as cryoprotectant.
NMR Spectroscopy:
Data Collection:
Data Processing: