RRAGC

PDB:3LLU

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:MGC cDNA library: AT20-A9:BC016668
Entry Clone Source:MGC
SGC Clone Accession:HPC09I-A06
Tag:mhhhhhhssgrenlyfq*g
Host:BL21-V2R-pRARE2

Construct

Prelude:RRAGC:S60-I237
Tag not removed
Even though the protein has been treated with TEV protease for two days, the tag-removed version is still minor in the mass spectrum. SDS-PAGE did not distinguish between cut and uncut versions. While the first visible residue is K61, and the protein was treated with Talon beads after Tev treatment (but in the presence of imidazole). In a parallel experiment, protein without treatment of TEV protease did not crystallize in the same time scale using the same condition. Another complication, the protein was crystallized in the presence of 1:100 (w/w) papain, which may further make the actual population of crystallized molecules unambiguous.
Sequence:

mhhhhhhssgrenlyfqgSKPRILLMGLRRSGKSSIQKVVFHKMSPNETLFLESTNKIYKDDISNSSFVNFQIWDFPGQMDFFDPTF
DYEMIFRGTGALIYVIDAQDDYMEALTRLHITVSKAYKVNPDMNFEVFIHKVDGLSDDHKIETQRDIHQRANDDLADAGLEKLHLSF
YLTSIYDHSIFEAFSKVVQKLI

Vector:pET28-MHL

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 mg/mL kanamycin and 25 mg/mL chloramphenicol at 37 degree. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 degree and the culutre 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 degree.

Purification

Procedure
The lysate was centrifuged at 15,000 rpm for 45 minutes and the supernatants were mixed with 5 mL 50% flurry of Talon Cobalt beads and incubated at 4 degree on rotary shaker for one hour. The mixture was then centrifuged at 2300 rpm for 5 min and the supernant 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 purifed by a Superdex-75 gel filtraton column pre-equilibrated with gel filtration buffer. Fractions containing the protein were collected and concentrated with Amicon Ultra-15 centrifugal filter. 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:22.5 mg/mL
Ligand
GNP, Mg2+**MassSpec:**native protein expected 22861.86 (uncut), 20725.56(uncut)
measured
uncut: 22862.49, 22918.56 (+56.08)
cut: 20727.25(minor) + 22919.63 (major)
Crystallization: Crystal used for structure deterination was grown in SGC-I condition A4 from initial screen.Crystal was grown in 2.0M (NH4)2SO4, 0.2M NaCl, 0.1M HEPES pH 7.5 in the presense of 1:100 (w/w) papain and GNP in sitting drop setup. Crystals grow to a mountable size within 3 months, only two crystals were seen in the drop.Cryo used 0.5 V well solution + 0.5 V 50% glycerol
NMR Spectroscopy:
Data Collection:
Data Processing: