IQGAP2

PDB:3IEZ

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source: Codon Devices Synthesized: SGC cDNA library: DNA 04-A9:IQGAP2
SGC Clone Accession:HPC09K-A06
Tag:N-terminal tag: mhhhhhhssgrenlyfq*g
Host:BL21-V2R-pRARE2

Construct

Prelude:IQGAP2:A1476-K1571
Tag not removed
Sequence:

```
mhhhhhhssgrenlyfqgAKPVKYTAAKLHEKGVLLDIDDLQTNQFKNVTFDIIATEDVGIFDVRSKFLGVEMEKVQLNIQDLLQMQ
YEGVAVMKMFDKVKVNVNLLIYLLNKK
```

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 degC 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 centrigued at 15,000 rpm for 45 minutes and the supernatants were mixed with 5 mL 50% flurry of Talon beads and incubated at 4 degC 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-trough was collected and 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%.

TEV protease failed to cleave the tag from the protein.

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 µL benzonase (Sigma Catalog # E1014, 250U/µL), and lysed using microfluidizer at 15,000 PSI.
Concentration:15.0 mg/mL
Ligand
MassSpec: Native expected 13211.33, measured 13211.83
Crystallization: Crystallization was setup using sitting drops with Red Wings and SGC-I screens initially. Crystals were seen from multiple conditions containing chymotrypsin, subtilisin, dispase or thermolysin, but not without protease in the same time frame.

Crystal used for refinement was grown in 30% PEG5000 MME, 0.2M (NH4)2SO4, 0.1M MES buffer pH 6.5, with 1:100 Chymotrypsin (w/w) in sitting drop setup. Cryoprotectant used paratone. Crystal derivative used for phasing was grown in 1.8 M (NH4)2SO4, 0.2 M NaAcetate, 0.1 M Hepes 7.5 in the presence of 1:100 dispase I or thermolysin (both drops have crystals but W.T. was not sure the exact drop used).
Crystals grow to a mountable size within one day.Crystal grown in the presence of chymotrypsin was checked by silver staining and only one polypeptide was found.
NMR Spectroscopy:
Data Collection:
Data Processing: