REM2

PDB:3CBQ

Revision

Revision Type:created
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Entry Clone Accession:BC035663
Entry Clone Source:MGC AT56-F8
SGC Clone Accession:HPC073-E12
Tag:mhhhhhhssgrenlyfq*g
Host:BL21-CodonPlus(DE3)-RIL

Construct

Prelude:
Sequence:

```
mhhhhhhssgrenlyfqgQKDGIFKVMLVGESGVGKSTLAGTFGGLQGDSAHEPENPEDTYERRIMVDKEEVTLVVYDIWEQGDAGG
WLRDHCLQTGDAFLIVFSVTDRRSFSKVPETLLRLRAGRPHHDLPVILVGNKSDLARSREVSLEEGRHLAGTLSCKHIETSAALHHN
TRELFEGAVRQIRLRRGRNHA
```

Vector:pET28-mhl (GI:134105571)

Growth

Medium: Terrific Broth
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 1.8 L of Terrific Broth medium in the presence of 50 µg/mL kanamycin and 25 µg/mL chloramphenicol at 37 °reeC. When OD600 reached ~3.0, the temperature of the medium was lowered to 15 µC 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 °C.

Purification

Procedure
The lysate was centrigued at 15,000 rpm for 45 minutes and the supernatants were loaded onto 5mL HiTrap HP column charged with Ni2+ ion. The column was washed steply using washing buffer containing 30, 60, and 500 mM imidazole. Fractions washed using 500 mM imidazole was collected and pooled together and further purifed by a Superdex-75 gel filtraton column pre-equilibrated with 20 mM HEPES buffer at pH 7.0, with 10 mM MgCl2 and 2 mM BME.

Fractions were collected and concentrated with Amicon Ultra-15 centrifugal filter. The purify of the preparation is tested by SDS-PAGE to be around 95%.

Extraction

Procedure
Frozen cells were thawed and resuspended in 150 mL extraction buffer with freshly added 0.5% CHAPS and 2mM BME, 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:38-40 mg/mL
Ligand
GDP, Mg2+**MassSpec:**Native: 21942.11, expected 21941.61
Crystallization:GDP were added to the concentrated protein to a final concentration of 5mM. Crystallization were set up using SGC-I and Red Wings screen with and without 1:100 different proteases. Crystal were seen for the following conditions:No protease: RW-G12
Chymotrypsin: SGC-B11
Trypsin: RW-B7, RW-A2
Clustered plates like crystals appear in the drop in one week.
Crystal used for structure determination were grown in Red Wings initial screen B07: 20.0% PEG3350, 0.2M KCl, no buffer in the mother liquor. Sitting drop vaporization. Cryo used: 20% PEG 3350 + 20% EG (Last updated YTONG 20080328)
NMR Spectroscopy:
Data Collection:
Data Processing: