SHPRH

PDB:4QN1

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:BC117686
Entry Clone Source:MGC CM31-E8
SGC Clone Accession:YTC014-B07
Tag:N-terminal His6-tag, removed
Host:BL21(DE3)V2R-pRARE2

Construct

Prelude:SHPRH:T1000-K1418

While the cDNA template BC117686 suggests a V to F mutation at position 1398,this mutation does not exist in the cloned construct, i.e. the sequenceis consistent with reference sequence NP_001036148.2 a.a. 1000-1418
Sequence:

gTMEELLTSLQKKCGTECEEAHRQLVCALNGLAGIHIIKGEYALAAELYREVLRSSEEHKGKLKTDSLQRLHATHNLMELLIARHPG
IPPTLRDGRLEEEAKQLREHYMSKCNTEVAEAQQALYPVQQTIHELQRKIHSNSPWWLNVIHRAIEFTIDEELVQRVRNEITSNYKQ
QTGKLSMSEKFRDCRGLQFLLTTQMEELNKCQKLVREAVKNLEGPPSRNVIESATVCHLRPARLPLNCCVFCKADELFTEYESKLFS
NTVKGQTAIFEEMIEDEEGLVDDRAPTTTRGLWAISETERSMKAILSFAKSHRFDVEFVDEGSTSMDLFEAWKKEYKLLHEYWMALR
NRVSAVDELAMATERLRVRDPREPKPNPPVLHIIEPHEVEQNRIKLLNDKAVATSQLQKKLGQLLYLTNLEK

Vector:pET28-MHL

Growth

Medium:
Antibiotics:
Procedure:LEX Bubbling

For native protein: The target protein was over-expressed in E. coli at 37degree C by inoculating 30 mL of overnight culture grown in Luria-Bertani medium into 2 L TerrificBroth medium in the presence of 50 ug/mL kanamycin and 34 ug/mL chloramphenicol. When the OD600 of the culture reached ~1.5, the temperature was lowered to 18 degree and the culture was induced with 0.25 mM final IPTG concentration. The cells were allowed to grow overnight before harvested by centrifugation (7,000 rpm Beckman JLA-8.1000rotor 15 min) and flash frozen in liquid nitrogen and stored at -80 degree.

For SeMet labelling, the target protein was over-expressed in E.coli using prepacked M9 SeMet growth media kit (Medicilon) following manufacturer's instruction. Harvested cells were flash frozen in liquid nitrogen and stored at -80 degree C.

Purification

Procedure
The lysate was centrifuged at 16,000 rpm (25,800xg RCF(average) for 60minutes. The supernant was supplemented with 6 mL Ni-NTA resin (50% flurry) and incubated on a rotary drum for 1 hour at 4 degree, then loaded onto Bio-rad gravity column.The beads deposited in the open column was then washed with 50 mL lysis buffer followed by 15 mL washing buffer. Bound proteins were eluted using 15 mL elution buffer. The N-terminal His-tag was reomoved by overnight incubation with TEV protease (1:30 w/w) at 4degree during dialysis against the dialysis buffer. Uncut proteins and TEVprotease were removed by passing the solution through 3mL Ni-NTA beads,andthe target protein was further purified by anion-exchange chromatography on a 5mL HiTrap Q column(GE Healthcare). The protein was further purified using gel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare)preequilibrated with gel filtration buffer. Fractions containing the targetprotein were pooled and concentrated by centrifugal filters (Amicon mwco30kDa). The yields of the native and SeMet proteins were about 4.5 mg and 9 mg per litre bacterialculture respectively, and the purity is above 95% judging from SDS-PAGE.

Extraction

Procedure
2L native cell pellet was resuspended in a total volume of 200 ml extraction buffer with1mM PMSF/Benzamidine freshly added and the cells disrupted by sonication for 10 mins at 5" on 7" off duty cycle at 120W output power.

4L SeMet cell pellet was resuspended in a total volume of 200 ml lysis buffer with1mM PMSF/Benzamidine freshly added and the cells disrupted by sonication for 10 mins at 5" on 7" off duty cycle at 120W output power
Concentration: Concentration used for crystallization : native protein: 18.1 mg/mL, SeMet protein: 20.9 mg/mL
Ligand
MassSpec:native protein: 48439.9 g/mol, expected 48439.9 g/mol.
Crystallization: In situ proteolysis: the native and SeMet proteins of SHPRH were mixed with

chymotrypsin at1:1000(w/w) ratio before crystallization. Crystals used for structure determination were grown at 298K in hanging drop setup by mixing 2 uL protein solution with 2 uL well solution containing 1.6Mammonium formate, 0.1 M Bis-Tris pH 5.5, 5% ethylene glycol.The crystals were cryoprotected by immersion in paratone-N before flash frozenin liquid nitrogen.

NMR Spectroscopy:

Data Collection:

Data Processing: