RC3H1

PDB:4YWQ
Entry Clone Accession:BC144408; SGC cDNA collection: 38-I5
Entry Clone Source:MGC
SGC Clone Accession:YTC019-B07
Tag:N-terminal His6-tag, removed
Host:BL21(DE3)-V2R-pRARE2
Vector:pET28-MHL
Prelude:RC3H1:M1-K445, no tag, treated with elastase

Sequence:

The actual sequence crystallized is a proteolytic fragment generated byelastase cleavag
e; while actual sequence is not confirmed, the following is themost probable sequence:
RSLGERTVTELILQHQNPQQLSSNLWAAVRARGCQFLGPAMQEEALKLVLLALEDGSALSRKVLVLFVVQRLEPRFPQASKTSIGHV
VQLLYRASCFKVTKRDEDSSLMQLKEEFRTYEALRREHDSQIVQIAMEAGLRIAPDQWSSLLYGDQSHKSHMQSIIDKLQTPA

Growth

Procedure:LEX Bubbling. For protein: The target protein was expressed in E. coli by inoculating 30 mL of overnight culture grown in Luria-Bertani medium into a 2 L of TerrificBroth medium in the presence of 50 ug/mL kanamycin and 34 ug/mLchloramphenicol at 37 degree. When the OD600 of the culture reached ~1.5, the temperature was lowered to 18 degree and protein overexpression was induced with 0.25 mM IPTG. The cells were allowed to grow overnight before harvested by centrifugation (7,000 rpm Beckman JLA-8.1000rotor 12min) and flash frozen in liquid nitrogen and stored at -80 degree.

Purification

Procedure: The lysate was centrifuged at 16,000 rpm (25,800xg RCF(average) for 60 minutes and each 200 mL supernatant was transferred to a Corning centrigue tube containing 6 mL Ni-NTA resinand incubated on a rotary drum for 1 hour at 4 degree, then loaded onto a Bio-rad open gravity column. The beads in the open column were then washed with 50 mL extraction buffer followed by with 20 mL washing buffer. Bound proteins were eluted using 15 mL elution buffer. The N-terminal His6-tag was reomoved by overnight incubation with TEV protease (1:20 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 cation-exchange chromatography on 5mL HiTrap S column(GE Healthcare). Then the protein was

further purified bygel filtration on a HighLoad 16/60 Superdex 200 column (GE Healthcare)preequilibrated with gel filtration buffer. Fractions containing targetprotein were pooled and concentrated by centrifugal filters (Amicon mwco30kDa). The final yield of the protein was about 12 mg per litre bacterialculture and the purity is above 99% judging from SDS-PAGE.

Extraction

Procedure: 2L native cell pellet was resuspended in a total volume of 400 ml extraction buffer with1 mM final concentration of PMSF/Benzamidine freshly added and the cells were disrupted by sonication for 10 mins at 5" on 7" off duty cycle at 120W output power,

Concentration:24.96 mg/ml

Structure Determination

MassSpec: native protein: 49959.1 g/mol, expected 49955.3 g/mol.
Crystallization: The protein was mixed with elastase at 1:1000 ratio (w/w) right before crystallization.Crystals were grown at 20 degrees using the sitting drop method by mixing 0.5 uL protein with 0.5 uL well solution consisting of 25% PEG8000, 0.2 M NaCl, 0.1 M Hepes pH 7.5, 5% ethyl glycol.The crystals were cryoprotected by immersion in well solution containing 15%ethylene glycol first, then immersion in Paratone-N.