ACVR1

PDB:4BGG

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:
Entry Clone Source: Site-directed mutagenesis
SGC Clone Accession:ACVR1A-c096
Tag:MGHHHHHHSSGVDLGTENLYFQ*SM. cleavable N-terminal hexahistidine tag.
Host:SF9 Spodoptera frugiperda Insect cells

Construct

Prelude:
Sequence:

MGHHHHHHSSGVDLGTENLYFQSMQRTVAR**D**ITLLECVGKGRYGEVWRGSWQGENVAVKIFSSRDEKSWFRETELYNTVMLRHENIL
GFIASDMTSRHSSTQLWLITHYHEMGSLYDYLQLTTLDTVSCLRIVLSIASGLAHLHIEIFGTQGKPAIAHRDLKSKNILVKKNGQC
CIADLGLAVMHSQSTNQLDVGNNPRVGTKRYMAPEVLDETIQVDCFDSYKRVDIWAFGLVLWEVARRMVSNGIVEDYKPPFYDVVPN
DPSFEDMRKVVCVDQQRPNIPNRWFSDPTLTSLAKLMKECWYQNPSARLTALRIKKTLTKIDEngineered **Q207D** mutation
in bold .

Vector:pFB-LIC-Bse

Growth

Medium: Sf9 cells at a density of 2x106/ml were infected with recombinant ACVR1 baculovirus (virus stock P3; 1ml of virus stock/1000 ml of cell culture). Cells were shaken at 110 rpm at 27Â°C in an Innova shaker. After 72 hours post-infection the cultures were harvested by centrifugation for 20min at 6000rpm. Cell pellets from each 1L flask were resuspended in 15 ml binding buffer (50 mM Hepes, pH 7.5; 500 mM NaCl; 5% Glycerol; 5 mM imidazole). Calbiochem protease inhibitor SET V was added to the cell suspension at a 1:2000 dilution and transferred to 50 ml tubes, and stored at -20Â°C.
Antibiotics:
Procedure:

Purification

Buffers
Procedure

Extraction

Buffers
Procedure
Extraction buffer, extraction method: The frozen cells were thawed and the volume increased to 80 ml with binding buffer. The cells were lysed by sonication over 12 min with the sonicator pulsing ON for 5 sec and OFF for 10 sec. The DNA was precipitated using 0.15% PEI (polyethyleneimine) pH 8. The cell lysate was spun down by centrifugation at 21.5K rpm at 4°C for 1 h. The supernatant was recovered for purification.Column 1: Ni-Affinity Chromatography. 6 ml of 50 % Ni-sepharose slurry was applied onto a 1.5 x 10 cm column. The column was equilibrated with binding buffer (25ml). Buffers: Binding buffer: 50 mM Hepes, pH 7.5; 500 mM NaCl; 5% Glycerol; 5 mM imidazole, 0.1mM TCEP Wash buffer: 50 mM Hepes, pH 7.5; 500 mM NaCl; 5% Glycerol; 25 mM imidazole, 0.1mM TCEPElution buffer: 50 mM HEPES, pH 7.5; 500 mM NaCl; 5% Glycerol; 50 to 250 mM imidazole, 0.1mM TCEPProcedure: The supernatant was applied by gravity flow onto the Ni-sepharose column. The bound protein was eluted by applying a step gradient of imidazole Â using 10 ml portions of elution buffer with increasing concentration of imidazole (50 mM, 100 mM, 150 mM, 250 mM). Enzymatic treatment: 0.1mg of TEV protease was added to the Ni-eluted protein to remove the tag.Column 2: Size Exclusion Chromatography Â S200 HiLoad 16/60 Superdex run on ÄKTA-ExpressBuffer: Gel Filtration buffer: 300 mM NaCl, 50 mM Hepes pH 7.5, 0.5mM TCEPProcedure: Prior to applying the protein, the S200 16/60 column was washed and equilibrated with gel filtration buffer. The protein was concentrated to 5 ml using an Amicon Ultra-15 filter with a 10 kDa cut-off. The concentrated protein was directly applied onto the equilibrated S200 16/60 column, and run at a flow-rate of 1 ml/min. The protein was eluted at 90-108 ml. Fractions containing the protein were pooled together. Column 3: Ni-Affinity Chromatography. 0.75 ml of 50 % Ni-sepharose slurry was applied onto a 1.5 x 10 cm column. The column was equilibrated with binding buffer (15ml).Buffers:Binding buffer: 50 mM Hepes, pH 7.5; 500 mM NaCl; 5% Glycerol; 5 mM imidazole, 0.1mM TCEP Wash buffer: 50 mM Hepes, pH 7.5; 500 mM NaCl; 5% Glycerol; 25 mM imidazole, 0.1mM TCEPElution buffer: 50 mM HEPES, pH 7.5; 500 mM NaCl; 5% Glycerol; 250 mM imidazole, 0.1mM TCEPProcedure: The cleaved protein was passed through the column followed by 3ml binding buffer. It was then washed with 8ml wash buffer. Anything remaining bound to the column was eluted with 15ml elution buffer.
Concentration:
Ligand
MassSpec: The purified protein was homogeneous and had an experimental mass of 34492.3 (after TEV cleavage), closely matching the expected mass 34492.7. Mass was determined by LC-MS, using an Agilent LC/MSD TOF system with reversed-phase HPLC coupled to electrospray ionisation and an orthogonal time-of-flight mass analyser. Proteins were desalted prior to mass spectrometry by rapid elution off a C3 column with a gradient of 5-95% isopropanol in water with 0.1% formic acid.
Crystallization: Protein was buffered in 50 mM HEPES pH 7.5, 300 mM NaCl, 10 mM DTT and 10mM L-arginine, 10 mM L-glutamate. The protein was concentrated to 10 mg/ml (calculated using an extinction co-efficient of 58900) in the presence of the inhibitor LDN-213844 (1 mM end concentration). Crystals were grown at 20°C in 150 nl sitting drops mixing 100 nl protein solution with 50 nl of a reservoir solution containing 0.2M ammonium citrate and 20% PEG 3350. On mounting crystals were cryoprotected with mother liquor plus 20% ethylene glycol before transfer to liquid nitrogen.
NMR Spectroscopy:
Data Collection: Resolution: 2.56 Ã resolutionX-ray source: Diamond Light Source, station I04-1
Data Processing: