

FDPS + minodronate

PDB:3B7L

Revision

Revision Type:created

Revised by:created

Revision Date:created

Entry Clone Accession:gi|4503685

Entry Clone Source:MGC

SGC Clone Accession:IMAGE:4132071

Tag:N-terminal tag: mgsshhhhhhsgrenlyfqghm

Host:BL21(DE3)

Construct

Prelude:

Sequence:

ghmNGDQNSDVTYAQEKQDFVQHFSQIVRVLTEDEMGHPEIGDAIARLKEVLEYNAIGGKYNRGLTVVAFRELVEPRKQDADSLQRA
WTVGWCVELLQAFFLVADDIMDSSLTRRGQICWYQKPGVGLDAINDANLLEACIYRLLKLYCREQPYYLNLIEFLQSSYQTEIGQT
LDLLTAPQGNVDLVRTEKRYKSIVKYKTAFYSFYLPIAAAMYMAGIDGEKEHANAKKILLEMGEFFQIQDDYLDLFGDPSVTGKIG
TDIQDNKCSWLVVQCLQRATPEQYQILKENYGQKEAKVARVKALYEELDPAVFLQYEEDSYSHIMALIEQYAAPLPPAVFLGLAR
KIYKRRK

Vector:p11

Growth

Medium:

Antibiotics:

Procedure:Overnight cultures in LB (10 ml with 100 µg/ml ampicillin) were used to inoculate 1 litre of LB medium containing 100 µg/ml ampicillin. Cultures were grown at 37°C until they reached an OD600 of 0.6-0.8 and then induced with 1 mM IPTG. The temperature was adjusted to 18°C and expression was allowed to continue overnight. The cells were collected by centrifugation.

Purification

Procedure

Column 1 : 2ml Ni-NTA agaroseApproximately 75mls of bacterial lysate was loaded by gravity onto a 2ml Ni-NTA agarose columns pre-equilibrated with binding buffer. The columns were then washed twice with 30ml binding buffer, then twice with 12.5ml of wash buffer. Protein was then eluted with 12.5 ml of elution buffer and collected as 1.5ml fractions. Fractions containing purified protein were pooled and concentrated to a volume of less than 5mls using a Vivaspin concentrator with 10 kD MW cutoff. Enzymatic treatment : His-tagged TEV protease was added

(50 μ g per mg FDPSA) and incubated at 4°C for 24 hours to remove the hexahistidine tag. The TEV protease and any uncleaved protein was removed by passing the solution over Ni-NTA agarose beads. The cleaved FDPSA was concentrated to 60mg/ml using a Vivaspin concentrator with 10 kD MW cutoff. Final protein buffer: 10 mM Hepes pH 7.5, 500 mM NaCl, 5% glycerol, 0.5 mM TCEP.Column 2 : Hiload 16/60 Superdex 200 prep grade 120 mlThe concentrated TEV cleaved protein was loaded onto the column at 1 ml/min using an AKTA purifier system. Eluted protein was collected in 1 ml fractions.

Extraction

Procedure

The cell pellet was resuspended in 50 mM HEPES pH 7.5, 5 mM imidazole, 500 mM NaCl, 5% glycerol and lysed using a high pressure cell disruptor. The lysate was centrifuged at 17,000 RPM for 30 minutes at 4°C and the supernatant was collected.

Concentration: All fractions containing pure protein were pooled. concentrated to 47.9mg/ml using a Vivaspin concentrator with 10 kD MW cutoff.

Ligand

minodronate**MassSpec:** Characterisation of the protein by mass spectrometry revealed MW of 42.96kDa uncleaved and 40.727kDa after TEV cleavage, coinciding with the predicted mass.

Crystallization: Minodronate was prepared as a 100 mM stock solution in 100mM Tris HCl pH 7.7. MgCl₂ was prepared as a 100mM aquaeus stock solution. Minodronate and MgCl₂ were added to the protein to a final concentration of 2 mM each and a final protein concentration of 15mg/ml. Crystals were grown at 20°C in 300 nl sitting drops by mixing 100 nl of protein solution and 200 nl of precipitant consisting of 0.2M NH₄Cl pH6.3, 20% PEG 6000, 10% Ethylene glycol. Crystals were mounted using 20% ethylene glycol as a cryoprotectant before flash freezing.

NMR Spectroscopy:

Data Collection: Resolution: 2.0 \AA ; X-ray source: Rotating anode, FR-E superbright.

Data Processing: