Cp-FDPS-Zoledronate

PDB:2HER

Revision

Revision Type:created
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Entry Clone Accession:cgd4_2550 (www.cryptodb.org)
Entry Clone Source: Cryptosporidium parvum Iowa strain gDNA
SGC Clone Accession:cgd4_2550:E38-L384; MAC017:C2
Tag:N-terminal: His6-tag with integrated TEV protease site: mgsshhhhhhssgrenlyfq*g
Host:E. coli BL21-(DE3)-R3

Construct

Prelude:
Sequence:

mgsshhhhhhssgrenlyfqgEYDYTDFINYYDKFKVIVYNVLKKLPLNDEIRKPVIEYYLNCIDYNVKKGKHIRGKILVLISSLSS
AYSNIKRDSIYLLGWVVEAIQALILIADDIMDSGKFRRGAPCWYIVHGQSNAINDIFFLKMLSLSLIFELSSVFGNDIVMKIQKIYN
ESIFFTVLGQHLDLSYFDLSKADKISERYFSMVEMKTSRYTFYMPVFFGLTLSEIQVSSAQLNLIEAILYKLGEFYQVHNDVSDYLF
NDSNADDICRFKLTWPLQKSFEIADEEMKLKISENYGKNSSLVKDCYNLLKINEHYLEYQRNALDYLIKLVKDITDDSLQKVFIHLI
HQISELITNSRSNADSNNSL

Vector:p15-tev-lic

Growth

Medium:
Antibiotics:
Procedure: Cp-FDPS was expressed in E. coli BL21-(DE3)-Rosetta-Oxford cells in Terrific Broth (TB) in the presence of ampicillin/chloramphenicol (50 microgram/mL and 25 microgram/mL respectively). A single colony was inoculated into 10 mL of LB with of ampicillin/chloramphenicol (50 microgram/mL and 25 microgram/mL respectively) in a 50 mL Falcon tube and incubated with shaking at 250 rpm overnight at 37 ºC. The culture was transferred into 50 mL of TB with 50 microgram/mL ampicillin in a 250 mL shaking flask and

incubated at 37 ºC for 3 hours. Then the culture was transfer into 1.8 L of TB with 50 microgram/mL kanamycin and 0.3 mL of antifoam (Sigma) in a 2 L bottle and cultured using the LEX system to an OD600 of ~5, cooled to 15 ºC, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 ºC.

Purification

Procedure
The cleared lysate was loaded onto a column prepacked with 10 g DE52 (Whatman) anion exchange resin (previously activated with 2.5 M NaCl and equilibrated with Binding Buffer); and subsequently onto a 1.0 - 2.5 mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer at approximately 1 - 1.5 mL/min. The volume of the Ni-NTA resin was pre-determined by the predicted protein yield from test expression analysis.
After the lysate was loaded, the DE52 was further washed with 20 mL of Binding Buffer. Each Ni-NTA column was then washed with 200 mL of Wash Buffer (50 mM HEPES pH 7.5, 500 mM NaCl, 30 mM imidazole, and 5 % glycerol) at 2 - 2.5 mL/min.
After washing, the protein was eluted with 15 mL of Elution Buffer (50 mM HEPES pH 7.5, 500 mM NaCl, 250 mM imidazole, and 5 % glycerol). EDTA was immediately added to the elution fraction to 1 mM; and DTT was added to 1-5 mM after approximately 15 more minutes.
The Ni-NTA purified protein was loaded onto a 26/60 S200 Superdex gel filtration column; and the major peak corresponding to the Cp-FDPS dimer was collected and concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). The concentrated protein was stored at 4 degC.

Stock concentration - 7.1 mg/mL.

Extraction

Procedure
The culture was harvested by centrifugation. Pellets from 4 L of culture were resuspended to approximately 40 mL/L of cell culture in Binding Buffer (50 mM HEPES pH 7.5, 500 mM NaCl, 5 mM imidazole, and 5 % glycerol) with the addition of protease inhibitors (1 mM benzamidine and 1 mM phenylmethyl sulfonyl fluoride (PMSF)). Resuspended pellets stored at -80 degC were thawed overnight at 4 degC on the day before purification. Prior to mechanical lysis, each pellet from 1 L of culture was pretreated with 0.5 % CHAPS and 500 units of benzonase for 40 minutes at room temperature. Cells were mechanically lysed with a microfluidizer (Microfluidizer Processor, M-110EH) at approximately 18000 psi; and the cell lysate was centrifuged using a Beckman JA-25.50 rotor at ~75000 x g (24000 rpms) for 20 minutes at 10 degC.
Concentration:
Ligand
MassSpec:
Crystallization: Purified Cp-FDPS was crystallized using the hanging drop vapor diffusion method in a VDXm plate with 350 µL of mother liquor at 18 ºC. 1.5 µL of the protein solution

(containing 10 mM Zoledronate, 10 mM isopentenyl pyrophosphate, and 10 mM MgCl2) was mixed with 1.5 µL of the reservoir solution (containing 1.6 M ammonium sulfate, 10 mM MgCl2, and 100 mM HEPES, pH 7.5). Crystals appeared after 4-5 weeks.

NMR Spectroscopy:

Data Collection:

Data Processing: