Cryptosporidium parvum cyclophilin cgd2_1660

PDB:2POE

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:cgd2_1660
Entry Clone Source: Cryptosporidum parvum Iowa strain genomic DNA
SGC Clone Accession:cgd2_1660:M1-D169; MAY01Y:A1
Tag:N-terminal: His6-tag with built-in TEV cleavage site (*): mhhhhhhssgrenlyfq*g
Host:BL21 (DE3)-R3/pRARE2

Construct

Prelude:
Sequence:

gMSVRIITNYGDLKFELFCSQCPKACKNFLALSASGYYKNTIFHKNIKGFIIQGGDPTGTGKGGESIYGRYFDDEIYPELKYDRRGI
LSMASKGASKKPNTNGSQFFITYSSLPQLNGEYVIFGKLIDGFETLNTLENCPSDKSHKPIDEIIIKDIVIHSNPIADQEILD

Vector:pET15-MHL

Growth

Medium:TB
Antibiotics:100 microG/mL ampicillin and 34 microG/mL chloramphenicol
Procedure:A single colony was inoculated into 10 mL of LB with of Antibiotics and incubated with shaking at 250 rpm overnight at 37 degC. The culture was transferred into 50 mL of TB with Antibiotics in a 250 mL shaking flask and incubated at 37 degC for 3 hours. The culture was then transferred into 1.8 L of above-specified growth medium with Antibiotics and 0.3 mL of antifoam (Sigma) in a 2L bottle and cultured using the LEX system to an OD600 of ~5, cooled to 15 degC and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 degC.

Purification

Procedure
The cleared lysate was loaded onto a 1.0-2.5 mL Ni-NTA (Qiagen) column (pre-equilibrated with Binding Buffer) at approximately 1.5-2.0 mL/min. The Ni-NTA column was then washed with 150 mL of Wash Buffer at 2-2.5 mL/min. After washing, the protein was eluted with Elution Buffer. DTT was added to 5 mM.

The cleared cell lysate was loaded onto a column containing 10 g DE-52 resin (Whatman) anion exchangeresin (previously activated with 2.5 M NaCl and equilibrated with Binding Buffer), and

then directly onto a 3 mL Ni-NTA (Qiagen) column at 1-1.5 mL/min. The volume of the Ni-NTA resin was pre-determined by the predicted protein yield from test expression analysis.When all the lysate was loaded, the DE52 column was further washed with 20 mL of Binding Bufferand the Ni-NTA column was washed with 200 mL of Wash Buffer at 2 mL/min. After washing, the protein was eluted from the Ni-NTA column with 15 mL of Elution Buffer. EDTA was added immediately to 1 mM.TCEP was added to 0.5 mM 15 minutes later.

The eluted protein was applied to a Sephadex S200 26/60 gel filtration column (GE Healthcare) pre-equilibrated with Gel Filtration Buffer.The collected fractions corresponding to the correct eluted protein peak were concentrated using a 15 mL Amicon Ultra centrifugal filter device from Millipore (10 kD cutoff). TCEP (5mM) was added to the concentrated protein. The protein sample identity were evalulated by mass spectroscopy. The concentrated sample (30 mg/ml) was stored at 4 degC.

Extraction

Procedure
Cells were resuspended to approximately 40 mL/L of cell culture in Binding Buffer with protease inhibitor (1 mM benzamidine-HCl 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 18,000 psi. The cell lysate was centrifuged at ~75,000 x g for 20 minutes at 10 degC.
Concentration:30 mg/mL
Ligand
MassSpec:
Crystallization: The protein was crystallized by means by sitting drop vapor diffusion in a 96-well Intelli-plate. The plate was set with 0.5 microL protein and 0.5 microL buffer in each drop, and 100 microL reservoir volume per well. Crystals grew in 5% PEG4000, HEPES, 10% PEG1000, 0.8M Na formate at pH 7.5 and 18 degC in two weeks.
NMR Spectroscopy:
Data Collection:
Data Processing: