Cp-CyP: Cryptosporidium parvum cyclophillin (cgd2_4120)

PDB:2PLU

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:cgd2_4120
Entry Clone Source: Cryptosporidium parvum strain Iowa genomic DNA
SGC Clone Accession:cgd2_4120:N3-V170; plate MAC01Y:E8
Tag:N-terminal His6-tag with integrated TEV cleavage site (*): mhhhhhhssgrenlyfq*g
Host:E. coli BL21-(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

gNPVVYFDISIGQTPAGRITMELFADKVPITAENFRALCTGEKGMGQSGKPLCYTGSFFHRIIPQFMIQGGDFTRGDGTGGESIYGS
KFRDENFVYTHDAPFLLSMANAGPNTNGSQFFITTVPCPWLDGKHVVFGKVLEGMEVVKSIEKCGSQNGKPTKSVCITASGV

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 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 1.0-2.5 mL Ni-NTA (Qiagen) column 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. The Ni-NTA column was then washed with 200 mL of Wash Buffer at 2 Â□ 2.5

mL/min. After washing, the protein was eluted with 15 mL of Elution Buffer. TCEP was added to 1-5 mM after approximately 15 more minutes.

The eluted protein from Ni-NTA was loaded onto a Sephadex S75 26/60 column equilibrated with Gel Filtration Buffer. The fractions from the peak eluting at the volume corresponding to monomeric protein were pooled and concentrated using a 15 mL Amicon Ultra centrifugal filter device from Millipore (10 kD cutoff). The protein sample identity and purity were evalulated by mass spectroscopy and 10% SDS-PAGE gel. The concentrated protein was flash frozen in liquid nitrogen and then stored at -80 degC.

Extraction

Procedure
Cells were resuspended to approximately 40 mL/L of cell culture in Binding Bufferwith 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 18000 psi; and the cell lysate was centrifuged using at ~75000 x g for 20 minutes at 10 degC.
Concentration:
Ligand
None for 2PLUCsA for 2POY**MassSpec:**
Crystallization: The native 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 350 microL reservoir volume per well. Crystals grew in 20% PEG 8000, 0.2M ammonium sulfate 0.1M sodium cacodylate, pH 5.5 at 18 degC.
For the CyP-CsA complex, cyclosporin A was added after gel filtration of the native protein at a ratio of 1:2. The mixture was then incubated at 4 degC under gental rocking overnight. After high speed centrifugation, the protein was concentrated for crystallization.Crystals formed in 25% w/v PEG 3350, 0.1M citric acid, pH 3.5 at 20 degC.
NMR Spectroscopy:
Data Collection:
Data Processing: