Cryptosporidium parvum calcium dependent protein kinase cgd7_1840

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Revision

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SGC Clone Accession:cgd7_1840:S197-L472; plate MAC024:D9
Tag:N-terminal His6-tag with integrated TEV cleavage site (*): mhhhhhhssgrenlyfq*g
Host:E. coli BL21-(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

mhhhhhhssgrenlyfqgSTKGDINQYYTLENTIGRGSWGEVKIAVQKGTRIRRAAKKIPKYFVEDVDRFKQEIEIMKSLDHPNIIR
LYETFEDNTDIYLVMELCTGGELFERVVHKRVFRESDAARIMKDVLSAVAYCHKLNVAHRDLKPENFLFLTDSPDSPLKLIDFGLAA
RFKPGKMMRTKVGTPYYVSPQVLEGLYGPECDEWSAGVMMYVLLCGYPPFSAPTDSEVMLKIREGTFTFPEKDWLNVSPQAESLIRR
LLTKSPKQRITSLQALEHEWFEKQLSSSPRNLL

Vector:p15-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 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 3 mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer at approximately 1 Â□ 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. MgCl2 and CaCl2 was added to the elution fraction to 1 mM; and TCEP was added to 2 mM after elutting from Ni-NTA column.

The sample was loaded onto a Sephadex S200 26/60 gel filtration column 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 (Millipore). TCEP (2mM), 1mM MgCl2, 2mM CaCl2 was added to the concentrated protein. The protein sample identity were evalulated by mass spectroscopyand found to be a mixture of different phosphorylated states.The protein was treated by lambda protein phosphatase from Biolabs.Subsequent evaluation by mass spectroscopy again revealed thatthe phosphatase resulted in a dominant species with a single phosphorylation site. The concentrated sample (20 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 ºC 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
MassSpec:
Crystallization: The protein was crystallized at 20 degC in 2.6 NaFormate, 0.2M Bis Tris propane using the sitting drop vapor diffusion method, with 1.3 microL of protein mixed with 1 microL of buffer.
NMR Spectroscopy:
Data Collection:
Data Processing: