Cp-ARFGAP: Cryptosporidium parvum ARFGAP (cgd5_1040)

PDB:2OWA

Revision

Revision Type:created
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Entry Clone Accession:cgd5_1040
Entry Clone Source:C. parvum strain Iowa genomic DNA
SGC Clone Accession:CP-PF08_0120:M1-E137; plate MAC01Z: F6
Tag: His6-tag with integrated TEV protease site: mhhhhhhssgrenlyfqg
Host:E. coli BL21-(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

gsMNISNLINADVDEKGFVSDKLRDNFFQIVRNRPENRTCFDCESRNPTWLSLSFAVFICLNCSSDHRKMGVHISFVRSSDLDKFTP
IQLVRMDIGGNGRARNYFKQVLGVNFSPKTKEYASSICGRQYKQILDSEISE

Vector:p15-tev-lic

Growth

Medium:TB
Antibiotics:
Procedure:A single colony was inoculated into 10 mL of LB with of ampicillin/chloramphenicol (100 microG/mL and 34 microG/mL respectively) and incubated with shaking at 250 rpm overnight at 37 degC. The culture was transferred into 50 mL of TB with ampicillin/chloramphenicol (100 microG/mL and 34 microG/mL respectively) 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 ampicillin/chloramphenicol (100 microG/mL and 34 microG/mL respectively) 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 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 (previously activated with 2.5 M NaCl and 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 eluted protein was applied to a Sephadex S75 26/60 gel filtration column pre-equilibrated with Gel Filtration Buffer. The fractions corresponding to the eluted protein peak were collected.

The eluted protein was treated with TEV protease in a molar ratio of protease:protein based on the measured activity of the available TEV,with the addition of 5 mM DTT and 15 mM imidazole, overnight at 4 degC. The cleaved protein was separated from the uncleaved protein by passage through another 2.5 mL Ni-NTA column. The His-tag was cleaved with TEV protease overnight at 4 degC in the presence of 1mM DTT. The cleaved sample was applied to a 1ml Ni-NTA column pre-equilibrated with Binding buffer. The flow-through was collected; and the column was rinsed with an additional 5 mL of Binding Buffer. These fractions were pooled and concentrated using a 15 mL Amicon Ultra centrifugal filter device from Millipore (15 kD cutoff). The concentrated protein was flash frozen and stored at -80 degC.

Extraction

Procedure
Cells were resuspended to approximately 40 mL/L of cell culture in Binding Buffer with the addition of protease inhibitors (1 mM benzamidine and 1 mM phenylmethyl sulfonyl fluoride (PMSF)). Resuspended pellets stored at -80degC were thawed overnight at 4degC on the day before purification. Prior to sonication, 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 sonicated using the ultrasonic liquid processor (Model Sonicator 3000 from MISONIX). The output level of the precessor was set to 8.0-8.5 which corresponds to the output power at about 100-120 watts. With cells sample sitting on the icy water, the processor was running in a programming mode with 10-second pulse-on and 10-second pulse-off alternatively for total 6 minutes processing time. The cell lysate was centrifuged using a Beckman JLA-16.250 rotor at ~38,400 x g (16,000 rpm) for 45 min at 4degC.
Concentration:12 mg/mL
Ligand
MassSpec:
Crystallization: The protein was crystallized by means by sitting drop vapor diffusion in a 96-well Intelliplate. The plate was set with 0.5 microL cleaved protein (12 mg/mL) with ZnCl2 added to 2 mM and 0.5 microL buffer in each drop, and 100 microL reservoir volume per well. Crystals emerged in 20% PEG3350 and 0.2 M ammonium formate at 18 degC after 10 days.
NMR Spectroscopy:
Data Collection:
Data Processing: