Crystal structure of Cryptosporidium parvum small GTPase RAB1A

PDB:2RHD

Revision

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Entry Clone Accession:cgd6_3220
Entry Clone Source:
SGC Clone Accession:cgd6_3220:M1-Q174:G9
Tag:mhhhhhhssgrenlyfqg
Host:E. coli BL21-(DE3)-R3-pRARE2

Construct

Prelude: Plasmodb
Sequence:

gMNPEYDYLFKLLLIGDSGVGKSCLLLRFADDTYTDSYISTIGVDFKIRTISLENKTVKLQIWDTAGQERFRTITSSYYRGAHGIII
VYDVTDRDSFDNVKQWIQEIDRYAMENVNKLLVGNKCDLVSKRVVTSDEGRELADSHGIKFIETSAKNAYNVEQAFHTMAGEIKKRV
Q

Vector:p15-mhl

Growth

Medium:TB
Antibiotics:
Procedure:cgd6_3220 was expressed in E. coli BL21-(DE3)-Rosetta-Oxford cells in Terrific Broth (TB) in the presence of ampicillin/chloramphenicol (50 microg/mL and 25 microg/mL respectively). A single colony was inoculated into 10 mL of LB with of ampicillin/chloramphenicol (50 microg/mL and 25 microg/mL respectively) in a 50 mL Falcon tube and incubated with shaking at 250 rpm overnight at 37 degC. The culture was transferred into 50 mL of TB with 50 microg/mL ampicillin in a 250 mL shaking flask and incubated at 37 degC for 3 hours. Then the culture was transfered into 1.8 L of TB with 50 microg/mL Chloramphenicol and 0.3 mL of antifoam (Sigma) in a 2 L bottle and cultured using the LEX system to an OD 600 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 Ni-NTA (Qiagen) column (pre-equilibrated with Binding

Buffer) at approximately 2Â□ 3 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 Ni-NTA column was 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. EDTA was immediately added to the eluted fraction to 1 mM; and 2mM TCEP was added approximately 15minutes later.

The eluted sample from Ni-NTA was loaded onto a Sephadex S75 26/60 column pre-equilibrated with Gel Filtration Buffer (10mM HEPES, pH7.5, 500mM NaCl).The fractions corresponding to the eluted protein peak were collected. The His-tag was cleaved with TEV protease for 3hr at room temperature in the presence of TCEP. Mass spectrometry results confirmed fully cleaved His-tag protein. The cleaved sample was applied on 1mL Ni-NTA column pre-equilibrated with Binding Buffer (no glycerol). The flow-through was collected and the column was rinsed with additional 5mL of binding buffer.The collected sample was concentrated using a 15 mL Amicon Ultra centrifugal filter device. The protein sample identity and purity were evalulated by mass spectroscopy and SDS-PAGE gel.The concentrated protein was stored at -80deg C.

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 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 lysed by sonicating for 10min and was centrifuged using a Beckman JA-16.25 rotor at 15,500 rpms for 45 minutes at 4 degC.
Concentration:
Ligand
5mMGDP5mM MgCl2 were added during concentrating the protein**MassSpec:**
Crystallization:
NMR Spectroscopy:
Data Collection:
Data Processing: