

Crystal structure of Plasmodium falciparum Rab11ain complex with GDP

PDB:3BFK

Revision

Revision Type:created

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Entry Clone Accession:PF13_0119

Entry Clone Source:

SGC Clone Accession:PF13_0119:D6-N185:D11

Tag:mgsshhhhhssgrenlyfq

Host:Ros-Ox

Construct

Prelude:

Sequence:

gDYYDYLFKIVLIGDSGVGKSNLLSRFTRDEFNLESKSTIGVEFATKSIQLKNNKIIKAQIWDTAGQERYRAITSAYYRGAVGALLV
YDITKKNSFENIEKWLKELRDNADSNIVILLVGNKSDLKHLRVINDNDATQYAKKEKLAFIETSALEATNVELAFHQLLNEIYNVRQ
KKQATKN

Vector:p15-mhl

Growth

Medium:TB

Antibiotics:

Procedure:The protein was expressed in *E. coli* BL21-(DE3)-Rosetta-Oxford cells in Terrific Broth (TB) in the presence of ampicillin/chloramphenicol (50microg/mL and 25 microg/mL respectively). A single colony was inoculated into 10 mL of LB with the same antibiotic mixture added in a 50 mL Falcon tube and incubated with shaking at 250 rpm overnight at 37 °C. The culture was transferred into 50 mL of TB with 50 ug/mL ampicillin in a 250 mL shaking flask and incubated at 37 °C for 3 hours. Then the culture was transferred into 1.8 L of TB with the same antibiotic mixture and 0.3 mL of antifoam (Sigma) added in a 2 L bottle and cultured using the LEX system to an OD₆₀₀ of ~5, cooled to 15 °C, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 °C.

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 1.0 × 2.5 mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer 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 × 2.5 mL/min. After washing, the protein was eluted with 15 mL of Elution Buffer. The protein was further purified by Size exclusion chromatography in a Superdex S75 26/60 column equilibrated with Gel Filtration Buffer. The fractions from the peak eluting at 197 mL corresponding to monomeric protein were pooled and the protein identity was evaluated by SDS-Page and mass spectroscopy.

The His6-tag was cleaved with TEV overnight at 4 degC and dialysed into Crystal Buffer (10 mM HEPES, pH 7.5, 500 mM NaCl). The cleaved sample was loaded onto a Ni-NTA column and the flow through was collected and concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). The protein sample identity and purity were evaluated by mass spectroscopy and SDS-PAGE gel. The concentrated protein was stored at 4 degC.

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 degrees Celsius were thawed overnight at 4 degrees Celsius 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 a Beckman JA-25.50 rotor at ~75000 x g (24000 rpms) for 20 minutes at 10 degrees Celsius.

Concentration:

Ligand

The protein was crystalized in presence of 1 mM MgCl₂ and 1 mM GDP.

MassSpec:
Crystallization: MAY5GV:D2-220% PEG 4000 0.2 M NH₄Acetate, 100 mM HEPES, pH 7.0, 20% Glycerol
The protein was crystallized at 20 degC in 13% PEG 4000 0.2 M NH₄Acetate, 100 mM Na Citrate, pH 7.5 using the hanging drop vapor diffusion method.

NMR Spectroscopy:

Data Collection:

Data Processing: