

Crystal Structure of Nucleolar GTP-binding protein 1 from Plasmodium Falciparum

PDB:2QU8

Revision

Revision Type:created

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Entry Clone Accession:PFF0625w

Entry Clone Source:

SGC Clone Accession:PFF0625w:L164-N370; plate MAC00G:G3

Tag:mgsshhhhhssgrenlyfq

Host:Ros-Ox

Construct

Prelude:

Sequence:

gLP SINPHKKTII LSGAPNVGKSSFMNIVSRANVDVQSYSF TTKNLVYGHFDHKL NKYQIIDTPGLLDRAFENRNTIEMTTITALAH
INGVILFIIDISEQCGLTIKEQINLFYSIKSVFSNKSIVIGFNKIDKCNMDSLSDNKL LKQILDNVKNPIKFSSFSTLTGVGVEQ
AKITACELLKNDQAESILLDQEQLLNTKLGETKN

Vector:p15-tv-lic

Growth

Medium:TB

Antibiotics:

Procedure:PFF0625w 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 transferred 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 2×3 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 2×2.5 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 DTT was added to 1mM after approximately 15 more minutes.

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 DTT. 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 evaluated 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

1mM GDP 2mM MgCl₂ were added during concentrating the protein

MassSpec:
Crystallization: MAY52M₂S:H1015%glycerol The protein was crystallized at 20 degC in 28%PEG2K MME, 0.1MBis-Tris pH6.5 using the sitting drop method.

NMR Spectroscopy:

Data Collection:

Data Processing: