Putative ubiquitin conjugating enzyme, PFE1350c, from Plasmodium falciparum

PDB:2R0J

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:PFE1350c
Entry Clone Source:
SGC Clone Accession:PFE1350c:I3-N150:D3
Tag:mhhhhhhssgrenlyfqg
Host: Ros-Ox

Construct

Prelude:
Sequence:

```
IPRRITKETQNLANEPPPGIMAVPVPENYRHFNILINGPDGTPYEGGTYKLELFLPEQYPMEPPKVRFLTKIYHPNIDKLGRICLDI
LKDKWSPALQIRTVLLSIQALLSSPEPDDPLDSKVAEHFKQDKNDAEHVARQWNKIYANNN
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Vector:p15-tev-lic

Growth

Medium:TB
Antibiotics:
Procedure:PFE1350c was expressed in E. coli BL21-(DE3)-Rosetta-Oxford cells in Terrific Broth meida in the presence of carbenicillin/chloramphenicol (100 microg/mL and 34 microg/mL respectively). A single colony was inoculated into 5 mL of LB with of carbenicillin/chloramphenicol (100 microg/mL and 34 microg/mL respectively) in a 14 mL round bottom tube and incubated with shaking at 250 rpm overnight at 37 degC. The culture (0.5 mL) was transferred into 50 mL of TB with carbenicillin and chloraphenicol in a 250 mL shaking flask and incubated at 37 degC overnight. The culture was then transferred into 2 X 1.8 L of TB with ampicillin and chloramphenicol and 0.3 mL of antifoam (Sigma) in 2 L bottles and cultured using the LEX system to an OD600 of ~5, cooled to 15 degC, and protein expression was induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 degC.

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 2 mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer at approximately 1 Â 1.5 mL/min. After the lysate was loaded, the DE52 was further washed with 10 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. EDTA was immediately added to the elution fraction to 1 mM; and DTT was added to 2 mM after 15 minutes.

The sample was loaded onto a Sephadex S200 26/60 column equilibrated with Gel Filtration Buffer. The fractions from the peak corresponding to monomeric protein were pooled and concentrated to 10 mg/mL using a 15 mL Amicon Ultra centrifugal filter device (Millipore). The protein sample identity and purity were evalulated by mass spectroscopy and SDS-PAGE. The concentrated protein was stored at -80 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 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 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 rpm) for 20 minutes at 10 degC.
Concentration:
Ligand
MassSpec:
Crystallization:MAY5GB:D4-23.5 M Sodium Formate, 0.1 M Bis-Tris, pH 6.0The protein was crystallized at 20 degC in 3.5 M Sodium Formate, 0.1 M Bis-Tris, pH 6.0 using the hanging drop vapour diffusion method.
NMR Spectroscopy:
Data Collection:
Data Processing: