Pv-UBC: Plasmodium vivax ubiquitin conjugating enzyme

PDB:2FO3

Revision

Revision Type:created
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Entry Clone Accession: Pv083175
Entry Clone Source: Plasmodium vivax Salvador I gDNA (kindly donated by John Barnwell of CDC)
SGC Clone Accession:PV-MAL13P1.227:K14-P138; plate MAC00H:D1
Tag:N-terminal: His6-tag with integrated TEV protease site: mgsshhhhhhssgrenlyfq*g
Host:E. coli BL21-(DE3)-CodonPlus-RIL from Stratagene

Construct

Prelude:
Sequence:

mgsshhhhhhssgrenlyfqgKYNMGNANYRIQKELHNFLNNPPINCTLDVHPNNIRIWIVKYVGLENTIYANEVYKLKIIFPDDYP
LKPPIVYFLQKPPKHTHVYSNGDICLSLLGDDYNPSLSISGLVLSIISMLSSAKEKKLP

Vector:p15TV-L

Growth

Medium: Terrific Broth (TB)
Antibiotics:50 microG/mL kanamycin and 25 microG/mL chloramphenicol
Procedure:A single colony was inoculated into 10 mL of LB with of Antibiotics and incubated with shaking at 250 rpm overnight at 37 degC. The culture was transferred into 50 mL of TB with Antibiotics 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 Antibiotics and 0.3 mL of antifoam (Sigma) in a 2L bottle and cultured using the LEX system to an OD600 of ~10, 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 column prepacked with 10 g DE52 (Whatman) anion exchange resin (previously activated with 2.5 M NaCl and equilibrated with Binding Buffer) and the DE52 column was further washed with 20 mL of Binding Buffer. The lysate was subsequently loaded onto a 1.0-2.5 mL Ni-NTA (Qiagen) column (pre-equilibrated with Binding Buffer) at approximately 1-1.5 mL/min. 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 elution fraction to 1 mM; and DTT was added to 1-5 mM after approximately 15 more minutes.

The eluted protein was applied to a Sephadex S200 26/60 gel filtration column pre-equilibrated with Gel Filtration Buffer. The fractions corresponding to the eluted protein peak were collected.

The His-tag was cleaved with TEV protease overnight at 4 degC in the presence of 1 mM 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 (5 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 protease inhibitor (1 mM benzamidine-HCl 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 18,000 psi. The cell lysate was centrifuged at ~75,000 x g for 20 minutes at 10 degC.
Concentration:6.5 mg/mL
Ligand
MassSpec:
Crystallization: The protein was crystallized by means of hanging drop vapor diffusion in a 24-well Intelli-Plate. The plate was set with 1.5 microL protein with NaI added to 20 mM and 1.5 microL buffer in each drop, and 300 microL reservoir volume per well. Crystals grew after 3 days in 22% PEG3350 and 0.19 M Ca(AC)2 at 18 degC.
NMR Spectroscopy:
Data Collection:
Data Processing: