PfMetAP1b

PDB:3S6B

Revision

Revision Type:created
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Entry Clone Accession:
Entry Clone Source:
SGC Clone Accession:PF10_0150:E168-N517:E7
Tag:MHHHHHHSSGRENLYFQG
Host:BL21-(DE3)-V2R-pRare2

Construct

Prelude:
Sequence:

EKEDHLKTIVKKHLSPENFDPTNRKYWVYDDHLKNFVNFKFTGDVRPWPLSKINHVPSHIERPDYAISSIPESELIYKRKSDIYVNN
EEEIQRIREACILGRKTLDYAHTLVSPGVTTDEIDRKVHEFIIKNNAYPSTLNYYKFPKSCCTSVNEIVCHGIPDYRPLKSGDIINI
DISVFYKGVHSDLNETYFVGDINDVPKEGKELVETCYFSLMEAIKKCKPGMFYKNIGTLIDAYVSKKNFSVVRSYSGHGVGKLFHSN
PTVPHFKKNKAVGIMKPGHVFTIEPMINQGHYSDVLWPDQWTSATSDGKLSAQFEHTLLITNNGVEILTKRTQDSPPLGFDTKDELY
YN

Vector:pET28-mhl

Growth

Medium:TB with kanamycin/chloramphenicol (50 microgram/mL and 34 microgram/mL, respectively)
Antibiotics:
Procedure:*Plasmodium falciparum*, methionine aminopeptidase 1b, PF10_0150, was expressed in *E. coli* BL21(λDE3) V2R pRare2 in growth medium. A single colony was inoculated into 25 mL of growth medium in a 50 mL Falcon tube and incubated with shaking at 250 rpm overnight at 37°C. Then the culture was transferred into 900 mL of growth medium with additional 0.3 mL of antifoam (Sigma), 9 mL of 0.83 M $MgSO_4$ and trace elements in a 1L bottle and cultured using the LEX system to an OD_{600} 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 2mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer at approximately 1 - 1.5 mL/min. 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. 1 mM TCEP and 1 mM EDTA was added to the eluted PF10_0150.

The sample was then loaded onto a superdex 200 gel filtration column. The eluted protein (in 10 mM Hepes, pH 7.5 and 500 mM NaCl) was concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore) with a 10 kDa cutoff. PP-MetAP (PF10_0150) was concentrated to 13.5 mg/ml and stored at 4°C.

Extraction

Procedure
The culture was harvested by centrifugation. Pellets from 2 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°C were thawed overnight at 4°C 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°C.
Concentration:
Ligand
MassSpec:
Crystallization: The protein was crystallized at 18°C in 27% PEG 3350, 0.2 M ammonium acetate, 0.1 M Tris pH 8.5 using the hanging drop method.
NMR Spectroscopy:
Data Collection:
Data Processing: