Pv-ASL

PDB:2HVG

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession: Pv003765
Entry Clone Source: Plasmodium vivax Salvador I genomic DNA
SGC Clone Accession:
Tag:N-terminal histag with thrombin protease cleavage site: mgsshhhhhhssglvprgs
Host:BL21(DE3)-R3

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsEHLKNISPIDGRYKKACGELSAFFSEHALIKHRIIVEVRWLLFLNEEELFFEKVTDHSVEVLNQIATN
ITDSDIARVKAIEEETNHDVKAVEYFVKEKLKNSKREDLLKIKEYVHYLCTSEDINNVAYATCLKACLNDVVIPCLEKIMLKLKDLA
VEYSHVPLLSRTHGQPASSTTFGKEMANFYARIHHHVGVIRRVKVCAKFNGAVGNFNAHKVASKDTDWVNTIGLFLKKHFNLTYSIY
CTQIQDHDYICELCDGLARANGTLIDLCVDIWLYISNNLLKLKVKEKEVGSSTMPHKVNPIDFENAEGNLHIANAFFKLFSSKLPTS
RLQRDLSDSTVLRNIGSSLAYCLIAYKSVLKGLNKIDIDRRNLEEELNQNWSTLAEPIQIVMKRHNYVDAYEELKQFTRGKVIDQKI
MQEFIKTKCAFLPQDVVDQLLELTPATYTGYADYLAKNVERLSGE

Vector:p28a-thrombin-LIC

Growth

Medium:
Antibiotics:
Procedure: Pv-ASL was expressed in E. coli BL21(DE3)-R3 in M9 SeMet HighÂ□Yield growth media (Medicilon) in the presence of ampicillin/chloramphenicol (100 microgram/mL and 34 microgram/mL, respectively). A single colony was inoculated into 50 mL of LB media with of ampicillin/chloramphenicol (100 microgram/mL and 34 microgram/mL respectively) in a 250mL flask and incubated with shaking at 250 rpm overnight at 37 ºC. The 50ml over night culture, was centrifuged at 2000 rpm and the cell pellets were washed with M9 SeMet High yield growth media. The resuspended pellet was added to 1.8 L of M9 SeMet High-Yield growth media with 100 microgram/mL ampicillin, 34 microgram/ml chloramphenicol and 0.3 mL of antifoam (Sigma) in a 2 L bottle and cultured using the LEX system to an OD600 around 1.5 . The inhibitory amino acid cocktail and SeMet was then added to the cultures and the temperature was lowered to 15 ºC. Twenty minutes later, the cultures were induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 ºC.

Purification

Procedure
Column 1: The cleared cell lysate was loaded onto a DE52(Whatman) column packed with 10 g of resin pre-equilibrated with binding buffer and subsequently onto a 2 mL Ni-NTA column (Qiagen) at approximately 1.5 mL/min. When all the lysate was loaded, 20 mL of Binding Buffer was added to the DE52 column. Then the Ni-NTA column was washed with 200 mL of Wash Buffer at 2 Â 2.5 mL/min. After washing, the protein was eluted from the Ni-NTA column with 15 mL of Elution Buffer. EDTA was added immediately to 1 mM; and DTT was added to 5 mM 15 minutes later.

Column 2: The eluted PV-PFB0295w::H6 was applied to a Sephadex S200 26/60 gel filtration column pre-equilibrated with 10 mM HEPES, pH 7.5 and 500 mM NaCl.The collected fractions corresponding to the eluted protein peak were concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore)with a 5 kDa cutoff.

Stock concentration: 18 mg/mL

Extraction

Procedure
The culture was harvested; and the cell pellet from 4 L of culture was suspended in 160 mL of binding buffer with protease inhibitors (1 mM benzamidine-HCl and 1 mM phenylmethyl sulfonyl fluoride, PMSF) and kept in 4 x 50 mL Falcon tube at ~ 80 ºC. Before purification, the cell pellet was thawed overnight at 4 ºC. Prior to mechanical lysis, each tube of cell suspension was pretreated with 0.5 % CHAPS and 500 units of benzonase (per 40 mL of resuspended cell pellet) for 40 minutes at room temperature. Then the cells were mechanically lysed in a microfluidizer (Microfluidizer Processor, M-110EH) pre-equilibrated in Binding Buffer at approximately 18000 psi. The lysate was centrifuged at 24,000 rpm for 20 minutes at 10 ºC.
Concentration:
Ligand
MassSpec:
Crystallization: Purified Pv-ASL was crystallized using the sitting drop vapor diffusion method in a VDX plate (Hampton Research) with 500 µL of mother liquor at 18 ºC. 1 µL of the protein solution was mixed with 0.75µL of the reservoir solution containing 1.7 M Ammonium sulfate, 0.2 M Potassium sodium tartrate, and 0.1 M Sodium citrate pH 5.6. Crystals appeared within 3 days.
NMR Spectroscopy:
Data Collection:
Data Processing: