Pv-GGPPS+GGPP

PDB:3CC9

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession: Pv092040
Entry Clone Source: Plasmodium vivax Salvador I genomic DNA
SGC Clone Accession: Pv-PF11_0295; plate MAC01Q:A12
Tag:N-terminal: His6-tag with integrated TEV protease site: mhhhhhhssgrenlyfq*g
Host:*E. coli* BL21-(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

mgsshhhhhhssgrenlyfqgMKETNSEEADSGLAFFRNMYDKYRDAFLSHLNEYSLEEEIKEHISKYYKLLFDYNCLGGKNNRGIL
VILIYEYVKNRDINSSEWEKAACLAWCIEILQAAFLVADDIMDKGEMRRNKYCWYLLKDVETKNAVNDVLLLYNSIYKLIEIYLRNE
SCYVDVIATFRDATLKTIIGQHLDTNIFSDKYSDAHREIDVNNINVPEQPVIDINMINFGVVYKNIVIHKTAYYSFFLPIVCGMLLAG
IAVDNLIYKKIEDISMLMGEYFQIHDDYLDIFGDSTKTGKVGSDIQNNKLTWPLIKTFELCSEPDKIKIVKNYGKNNLACVKVIDSL
YEQYKIRKHYESYEKAQKAKILSAINELHHEGIEYVLKYLLEILFTGV

Vector:p15-tev-lic

Growth

Medium:TB
Antibiotics:
Procedure:A single colony was inoculated into 10 mL of LB with of Antibiotics and incubated with shaking at 250 rpm overnight at 37 ºC. The culture was transferred into 50 mL of TB with Antibiotics in a 250 mL shaking flask and incubated at 37 ºC 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 2 L bottle and cultured using the LEX system to an OD600 of ~5, cooled to 15 ºC and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 ºC.

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 1.0 Â□ 2.5 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 20 mL of Binding Buffer. Each 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 1 Â□ 5 mM after approximately 15 more minutes. The sample was loaded onto 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 correct eluted protein peak were concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). The protein sample identity and purity were evalulated by mass spectroscopy and SDS-PAGE gel. The concentrated sample was stored at 4 °C.

Extraction

Procedure
Cells 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 ºC were thawed overnight at 4 ºC 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 ºC.
Concentration:12.1 mg/mL
Ligand
MassSpec:
Crystallization:1.5 microL of protein (at 12.1 mg/mL in 10 mM HEPES, pH 7.5, 500 mM NaCl containing 1 mM GGPP and 2 mM MgCl2) was mixed with 1.5 microL of reservoir solution consisting of 25% PEG 3350, 200 mM (NH4)2SO4, 100 mM Tris, pH 8.5 and incubated in hanging drops over 350 microL of reservoir solution at 18 °C. Crystals appeared overnight.
NMR Spectroscopy:
Data Collection:
Data Processing: