Pv-FDPS: Plasmodium vivax farnesyl diphosphate synthase

PDB:2IHI

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
SCYVDVIATFRDATLKTIIGQHLDTNIFSDKYSDAHREIDVNNINVPEQPVIDINMINFGVYKNIVIHKTAYYSFFLPIVCGMLLAG
IAVDNLIYKKIEDISMLMGEYFQIHDDYLDIFGDSTKTGKVGSDIQNNKLTWPLIKTFELCSEPDKIKIVKNYGKNNLACVKVIDSL
YEQYKIRKHYESYEKAQKAKILSAINELHHEGIEYVLKYLLEILFTGV

Vector:p15-tev-lic

Growth

Medium:
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 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 2 L bottle and cultured using the LEX system to an OD600 of ~5, 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 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 (50 mM HEPES pH 7.5, 500 mM NaCl, 30 mM imidazole, and 5 % glycerol) at 2 Â□ 2.5 mL/min. After washing, the protein was eluted with 15 mL of Elution Buffer (50 mM HEPES pH 7.5, 500 mM NaCl, 250 mM imidazole, and 5 % glycerol). 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 oC.

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 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 rpms) for 20 minutes at 10 oC.
Concentration:16.5 mg/mL for Pv-FDPS with His6-tag.
Ligand
MassSpec:
Crystallization: The native protein was crystallized by means by hanging drop vapor diffusion in a VDXm plate. The plate was set with 1.5 microL protein and 1.5 microL buffer in each drop, and 350 microL reservoir volume per well. Crystals grew overnight in 22% Peg 3350, 200 mM Li2SO4, 100 mM Tris, pH 8.5 at 18 degC.
NMR Spectroscopy:
Data Collection:
Data Processing: