Pb-OMPDC: Plasmodium orotidine 5

PDB:2FDS

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:GI:68074385
Entry Clone Source:*Plasmodium berghei* genomic DNA
SGC Clone Accession:PBG-PF10_0225:; plate M:E7
Tag:N-terminal: His6-tag with integrated thrombin protease site: mgsshhhhhhssglvpr*gs
Host:E. coli BL21-(DE3)-CodonPlus-RIL from Stratagene

Construct

Prelude:
Sequence:

```
mgsshhhhhhssglvprgsHFKTKLKNRRNEVNTCLCIGLDPDEDDIKNFMRNEEKNGYKNVKNNMNSNNNRIENVIKIGKEILLTD
EENIENLSEEDKFFYFFNHFCFYIINNTKEYALIYKMNFAFYIPYGSVGINALKNVFDYLNSMNIPTMLDMKINDIGNTVKNYRKFI
FEYLKSDSCTINVYMGTSMLKDICFDYEKNKYYSAYVLIKTTNKDSFIFQNELSINDKQAYIVMAEETQKMATDLKIDQNNEFIGFV
VGSNAFEEMKIIRNKFPDSYILSPGIGAQNGDLYKTLKNGYNKDYEKLLINVGRAITKSPNPKKSSESYYNQIIQIFKDIENGGNIE
QVYL
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Vector:p28a-LIC

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 ~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 1.0-2.5 mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer at approximately 1-1.5 mL/min. The volume of the Ni-NTA resin was pre-determined by the predicted protein yield from test expression analysis. 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 2-2.5 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 protein was dialyzed overnight against Crystal Buffer and finally concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). The concentrated sample was flash frozen in N2(l) and stored at -80 ºC.

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:74 mg/mL
Ligand
MassSpec:
Crystallization: The protein was crystallized by means of hanging drop vapor diffusion in a 24-well Linbro 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 overnight in 16% PEG 3350 and 140 mM diammonium hydrogen citrate at 18 degC.
NMR Spectroscopy:
Data Collection:
Data Processing: