Plasmodium Knowlesi Choline Kinase, PKH_134520

PDB:3C5I

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession: Pkn-PF14_0020
Entry Clone Source:
SGC Clone Accession: Pkn-PF14_0020:P70-D437:F1
Tag:mgsshhhhhhssgrenlyfq
Host: Ros-Ox

Construct

Prelude:wrong sequence in NCBI - use PlasmoDB
Sequence:

gPLCAQEFSDLTDPLYIKKICLEKVPEWNHFTEDNLRVKQILSGLTNQLFEVGLKEETANNYNSIRTRVLFRIYGKHVDELYNTISE
FEVYKTMSKYKIAPQLLNTFNGGRIEEWLYGDPLRIDDLKNPTILIGIANVLGKFHTLSRKRHLPEHWDRTPCIFKMMEKWKNQLFK
YKNIEKYNCDIHKYIKESDKFIKFMKVYSKSDNLANTIVFCHNDLQENNIINTNKCLRLIDFEYSGFNFLATDIANFFIETSIDYSV
SSYPFFEIDKKKYISYENRKLFITAYLSNYLDKSLVVPTPKLIDEILEAVEVQALGAHLLWGFWSIIRGYQTKSYNEFDFFLYAEQR
LKMYDDQKEYLISNNIIKGYD

Vector:p15-tev-lic

Growth

Medium:TB
Antibiotics:
Procedure: The protein was expressed in E. coli BL21-(DE3)-Rosetta-Oxford cells in Terrific Broth (TB) in the presence of ampicillin/chloramphenicol (50 microg/mL and 25 microg/mL respectively). A single colony was inoculated into 10 mL of LB with of ampicillin/chloramphenicol (50 microg/mL and 25 microg/mL respectively) in a 50 mL Falcon tube and incubated with shaking at 250 rpm overnight at 37 degC. The culture was transferred into 50 mL of TB with 50 microg/mL ampicillin in a 250 mL shaking flask and incubated at 37 degC for 3 hours. Then the culture was transfered into 1.8 L of TB with 50 microg/mL kanamycin and 0.3 mL of antifoam (Sigma) in a 2 L bottle and cultured using the LEX system to an OD 600 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. 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. The 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. TCEP was added to 1mM after approximately 15 more minutes.

The His6-tag was cleaved with TEV overnight at 4 degC in the presence of 1 mM TCEP and dialysed into Crystal Buffer (10 mM HEPES, pH 7.5, 500 mM NaCl). The cleaved sample was passed through a second Ni-NTA column before being loaded onto a Sephadex S200 26/60 column equilibrated with Gel Filtration Buffer. The fractions from the peak corresponding to monomeric protein were pooled and 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 protein was flash frozen in liquid nitrogen and then froze at -80 degC.

Extraction

Procedure
The culture was harvested by centrifugation. Pellets from 4 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 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 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 degC.
Concentration:
Ligand
MassSpec:
Crystallization: The protein was crystallized at 20 degC in 15% PEG 8000 0.2M Magnesium chloride 0.1M Tris pH - 8.5 using the sitting drop vapor diffusion method.
NMR Spectroscopy:
Data Collection:
Data Processing: