Py-OAT: Plasmodium yoelii ornithine aminotransferase (PY00104)

PDB:1Z7D

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:PY00104
Entry Clone Source: Plasmodium yoelii 17XNL genomic DNA
SGC Clone Accession:PY00104:; plate 2001:G10
Tag: His-tag with integrated thrombin protease site: mgsshhhhhhssglvprgs
Host:E.coli BL21 (Gold)

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsMEFVKDLKTPEDYINNELKYGAHNYDPIPVVLKRAKGVFVYDVNDKRYYDFLSAYSSVNQGHCHPNIL
NAMINQAKNLTICSRAFFSVPLGICERYLTNLLGYDKVLMMNTGAEANETAYKLCRKWGYEVKKIPENMAKIVVCKNNFSGRTLGCI
SASTTKKCTSNFGPFAPQFSKVPYDDLEALEEELKDPNVCAFIVEPIQGEAGVIVPSDNYLQGVYDICKKYNVLFVADEVQTGLGRT
GKLLCVHHYNVKPDVILLGKALSGGHYPISAVLANDDImLVIKPGEHGSTYGGNPLAASICVEALNVLINEKLCENAEKLGGPFLEN
LKRELKDSKIVRDVRGKGLLCAIEFKNELVNVLDICLKLKENGLITRDVHDKTIRLTPPLCITKEQLDECTEIIVKTVKFFDERF

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 50 microG/mL kanamycin 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 50 microgG/mL kanamycin and 0.3 mL of antifoam (Sigma) in a 2 L bottle and cultured using the LEX system to an OD600 of ~1, cooled to 15 degC and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 degC.

Purification

Procedure
The cleared cell lysate was loaded onto a DE52 (Whatman) column packed with 10 g of resin and subsequently onto a 2.5 mL Ni-NTA column at approximately 1.5 mL/min. When all the lysate

was loaded, 20 mL of Binding Buffer was added to the DE52 column. Then each Ni-NTA column was washed with 200 mL of Wash Buffer at 2-2.5 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 1 mM 15 minutes later. The eluted protein was transferred into a dialysis bag from Pierce (5 kD cutoff) and dialyzed against 1 L of overnight at 4 degC. The next morning, the protein was transferred out of the dialysis bag and concentrated using 15 mL Amicon Ultra centrifugal filter device from Millipore (5 kD cutoff).

Extraction

Procedure
Cells were centrifuged and the cell pellets were resuspended in binding buffer with protease inhibitor (1 mM benzamidine-HCl and 1 mM phenylmethyl sulfonyl fluoride, PMSF) and flash frozen. The thawed cell pellet was lysed by a combination of 0.5% CHAPS (Sigma) and sonication (1x 30 sec). Lysate was cleared by centrifugation at 35,000 x g and passed through DE52 from Whatman in 0.5 M NaCl.
Concentration:15 mg/mL
Ligand
MassSpec:
Crystallization: The protein was crystallized by means by hanging drop vapor diffusion in a 24-well Linbro plate. The plate was set with 1.5 microL protein (15 mg/mL) and 1.5 microL buffer in each drop, and 500 microL reservoir volume per well. Crystals emerged in 20% PEG 3350, 0.2 M Na dihydrogen phosphate at 20 degC.
NMR Spectroscopy:
Data Collection:
Data Processing: