Py-MDR2: Plasmodium yoelii Multi-Drug Resistance 2

PDB:2GHI

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:XP_726637
Entry Clone Source: Plasmodium yoelii 17XL
SGC Clone Accession:
Tag:N-terminal: His6-tag with integrated TEV protease site: mgsshhhhhhssgrenlyfq*g
Host:E. coli BL21-(DE3)-CodonPlus-RIL from Stratagene

Construct

Prelude: Sequence after cutting tag:
Sequence:

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gLESFSLTSHEKKFGVNIEFSDVNFSYPKQTNHRTLKSINFFIPSGTTCALVGHTGSGKSTIAKLLYRFYDAEGDIKIGGKNVNKYN
RNSIRSIIGIVPQDTILFNETIKYNILYGKLDATDEEVIKATKSAQLYDFIEALPKKWDTIVGNKGMKLSGGERQRIAIARCLLKDP
KIVIFDEATSSLDSKTEYLFQKAVEDLRKNRTLIIIAHRLSTISSAESIILLNKGKIVEKGTHKDLLKLNGEYAEMWNMQSGGNDI
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Vector:pET28a-LIC

Growth

Medium: Terrific Broth (TB)
Antibiotics:50 µg/mL kanamycin and 25 µg/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 ~10, 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. 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 His6-tag was cleaved with TEV overnight at 4 ºC in the presence of 5 mM DTT and dialyzed overnight against 10 mM HEPES, pH 7.5 and 500 mM NaCl. The cleaved sample was applied to a 2 mL Ni-NTA column pre-equilibrated with 10 mM HEPES, pH 7.5, 500 mM NaCl, and 15 mM imidazole. Imidazole was added to the cleaved Py-MDR2 sample to 15 mM and applied to the Ni-NTA column. The flow-through was collected; and the column was rinsed was an additional 5 mL of 10 mM HEPES, pH 7.5, 100 mM NaCl, and 15 mM imidazole. These fractions were pooled and concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). The concentrated protein was stored at 4 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:16.6 mg/mL for His6-tag cleaved protein
Ligand
MassSpec:
Crystallization: Purified Py-MDR2 was crystallized using the hanging drop vapor diffusion method in a VDXm plate with 350 µL of mother liquor at 18 ºC. 1.5 µL of the protein solution was mixed with 1.5 µL of the reservoir solution containing 29 % PEG 3350, 4 % glycerol, 200 mM LiSO4, and 100 mM BisTris propane, pH 5.5. Crystals appeared after 2 Â 3 weeks.
NMR Spectroscopy:
Data Collection:
Data Processing: