Py-U5-15kD: Plasmodium yoelii U5 small-nuclear-ribonucleoprotein-particle-specific 15 kD protein (thioredoxin like protein 4A)

PDB:2AV4

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:PY07357
Entry Clone Source:*Plasmodium yoelii* 17XNL genomic DNA
SGC Clone Accession:PY07357:; plate MAC007:H9
Tag:N-terminal: His-tag with integrated thrombin protease site: MGSSHHHHHHSSGLVPR*GS
Host:E. coli BL21-(DE3)-CodonPlus-RIL from Stratagene

Construct

Prelude:
Sequence:

mgsshhhhhhssglvprgsSFMLQHLNSGWAVDQAIVNEDERLVCIRFGHDYDPDCMKMDELLYKVADDIKNFCVIYLVDITEVPDF
NTMYELYDPVSVMFFYRNKHMMIDLGTGNNNKINWPMNNKQEFIDIVETIFRGARKGRGLVISPKDYSTKYKY

Vector:pET28a-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 at least 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 cell lysate was loaded onto a column containing 10 g DE-52 resin (Whatman) anion exchangeresin (previously activated with 2.5 M NaCl and equilibrated with Binding Buffer), and then directly onto a 2.5 mL Ni-NTA (Qiagen) 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 the 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.DTT was added to 5 mM 15 minutes later.

The eluted protein was applied to a Sephadex S200 26/60 gel filtration column pre-equilibrated with Gel Filtration Buffer. The collected fractions corresponding to the eluted protein peak were concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). The concentrated sample was flash frozen in N2(l) and stored at -80 degC.

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, the resuspended culture was pre-treated with 0.5% CHAPS and 500 units of benzonase (per 40 mL of resuspended culture) 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:7.5 mg/mL
Ligand
MassSpec:
Crystallization: The protein was crystallized by means by hanging drop vapor diffusion in a Linbro plate. The plate was set with 1.5 microL uncleaved protein (27 mg/mL) and 1.5 microL buffer in each drop, and 300 microL reservoir volume per well. Crystals grew overnight in 1.4 M NaCl and 100 mM Tris and pH 9.0 at 18 degC.
NMR Spectroscopy:
Data Collection:
Data Processing: