Crystal structure of Plasmodium falciparum thioredoxin, PFI0790w

PDB:3CXG

Revision

Revision Type:created
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Entry Clone Accession:PFI0790w
Entry Clone Source:
SGC Clone Accession:PFI0790w:Q18-K132:E1
Tag:mgsshhhhhhssgrenlyfq
Host: Ros-Ox

Construct

Prelude:
Sequence:

mhhhhhhssgrenlyfqgQSIYIELKNTGSLNQVFSSTQNSSIVIKFGAVWCKPCNKIKEYFKNQLNYYYVTLVDIDVDIHPKLNDQ
HNIKALPTFEFYFNLNNEWVLVHTVEGANQNDIEKAFQKYCLEKAK

Vector:p15-mhl

Growth

Medium:TB
Antibiotics:
Procedure: The protein was expressed in *E. coli* BL21-(DE3)-Rosetta-Oxford cells inTerrific Broth (TB) in the presence of ampicillin/chloramphenicol (50microg/mL and 25 microg/mL respectively). A single colony was inoculatedinto 10 mL of LB with the same antibiotic mixture added in a 50 mL Falcontube and incubated with shaking at 250 rpm overnight at 37 °C. The culturewas transferred into 50 mL of TB with 50 ug/mL ampicillin in a 250 mLshaking flask and incubated at 37 °C for 3 hours. Then the culture wastransferred into 1.8 L of TB with the same antibiotic mixture and 0.3 mL ofantifoam (Sigma) added in a 2 L bottle and cultured using the LEX system toan OD_{600} of ~5, cooled to 15 °C, and induced with 0.5 mMisopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 °C.

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 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. The 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. The protein was further purified by Size exclusion chromatography in a Superdex S75 26/60 column equilibrated with Gel Filtration Buffer. The fractions from the peak eluting at 199 mL corresponding to monomeric protein were pooled and the protein identity was evaluated by SDS-Page and mass spectroscopy.

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 degrees Celsius were thawed overnight at 4 degrees Celsius 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 JLA-16.25 rotor at 15000 rpms for 1 hour at 4 degree Celsius.
Concentration:
Ligand
MassSpec:
Crystallization:MAY5WV:G4-12M AmmSO4 0.2M NaAcetate 0.1M Hepes pH 7.5 5%MPD, 15% Glycerol
NMR Spectroscopy:
Data Collection:
Data Processing: