Pf-Clp: Plasmodium falciparum ClpP protease

PDB:2F6I

Revision

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Entry Clone Accession:PFC0310c
Entry Clone Source:*Plasmodium falciparum* 3D7 gDNA
SGC Clone Accession:PFC0310c:D179-K370; plate MAC2004:B3
Tag:N-terminal His-tag with integrated Thrombin protease site:
MHHHHHHSSGVDLGTENLYFQ*sm
Host:E. coli BL21-(DE3)-CodonPlus-RIL from Stratagene

Construct

Prelude:
Sequence:

mhhhhhhssgvdlgtenlyfqsmDIKDMKKDVKLFFFKKRIIYLTDEINKKTADELISQLLYLDNINHNDIKIYINSPGGSINEGLA
ILDIFNYIKSDIQTISFGLVASMASVILASGKKGKRKSLPNCRIMIHQPLGNAFGHPQDIEIQTKEILYLKKLLYHYLSSFTNQTVE
TIEKDSDRDYYMNALEAKQYGIIDEVIETKLPHPYFNKVEK

Vector:pET21a-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 2.5, 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 DE52 (Whatman) column packed with 10 g of resin (previously activated with 3 M NaCl and equilibrated with Binding Buffer), and subsequently onto a 2.5 mL Ni-NTA column at approximately 1.5 mL/min. When all the lysate was loaded, both columns were washed with 20 mL of Binding Buffer. Then the Ni-NTA column was washed with 200 mL of Wash Buffer at 2Â□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 then added to 1 mM 15 minutes later.

The protein was dialysed overnight in a dialysis cassette (Pierce) in Crystal Buffer. The following day, it was concentrated using a 15 mL Amicon Ultra centrifugal filter device Millipore (5 kD cutoff).Protein concentration was estimated by means of absorbance at OD280. Aliquots of the purified protein were flash frozen in N2(l) and stored at -80°C.

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:30 mg/mL
Ligand
MassSpec:
Crystallization: The protein was crystallized by means of hanging drop vapor diffusion in a 24-well Linbro Plate. The plate was set with 1.5 microL protein and 1.5 microL buffer in each drop, and 500 microL reservoir volume per well. Crystals grew in 100 mM cacodylate buffer, pH 7.0, 200 mM ammonium sulfate and 23% PEG MME 550 at 18 degC.
NMR Spectroscopy:
Data Collection:
Data Processing: