Tg-ACP: Toxoplasma gondii apicoplast-targeted acyl carrier protein

PDB:2QNW

Revision

Revision Type:created
Revised by:created
Revision Date:created
Entry Clone Accession:55.m00019
Entry Clone Source:
SGC Clone Accession:55.m00019:s:S100-S180; plate MAC02B:E12
Tag:N-terminal His6-tag with integrated TEV cleavage site (*): mhhhhhhssgrenlyfq*g
Host:E. coli BL21-(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

gSSDDRPLLERVKDVVADQLGVDRARINPESNFIKDLDADSLDSVELVMAFEEKFGVSIPDEEASKIATVQDALSYIEKAKS

Vector:p15TV-LIC

Growth

Medium:TB
Antibiotics:100 microG/mL ampicillin and 34 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 ~5, cooled to 15 degC and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 ºC.

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 mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer at approximately 1 Â□ 1.5 mL/min. After the lysate was loaded, the DE52 was further washed with 10 mL of Binding Buffer. The Ni-NTA column was then washed with 200 mL of Wash Buffer at 2 Â□ 2.5 mL/min. After washing, the protein was eluted with 15 mL of Elution Buffer. EDTA

was immediately added to the elution fraction to 1 mM; and DTT was added to 2 mM after 15 minutes.

The sample was incubated with a 1:20 molar equivalent of TEV protease overnight at 4 degC while being dialyzed into buffer containing 10 mM HEPES pH 7.5, 500 mM NaCl, 10 mM imidazole, and 1 mM DTT. The cleaved 55.m00019 protein was separated from the cleaved tag, uncleaved protein, and TEV protease by passage through another 2 mL Ni-NTA resin. The sample was then subjected to buffer exchange by repeated dilution and concentration (final buffer 10 mM HEPES pH 7.5, 500 mM NaCl) and concentrated to 26 mg/mL. Zinc acetate was added to 100 mM immediately before crystallization setup, which resulted in some precipitation of the protein which was removed by centrifugation.

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 ºC 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 at ~75000 x g for 20 minutes at 10 degC.
Concentration:
Ligand
MassSpec:
Crystallization: The protein was crystallized at 20 degC in 30% PEG 1500, with 0.3 microL buffer added to 0.3 microL protein using the sitting drop vapour diffusion method.
NMR Spectroscopy:
Data Collection:
Data Processing: