Cp 14-3-3: Cryptosporidium parvum 14-3-3 protein

PDB:2O8P

Revision

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Entry Clone Accession:cgd7_2470
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SGC Clone Accession:cgd7_2470:E6-K238; MAC01E:E4
Tag:
Host:E. coli BL21-(DE3)-R3-pRARE2

Construct

Prelude:
Sequence:

gEMDERLLQKYRAQVFEWGGCFDKMFEALKSLIYLSEFENSEFDDEERHLLTLCIKHKISDYRTMTSQVLQEQTKQLNNDELVKICS
EYVFSLRKDIKAFLQSFEDCVDRLVEKSFFSKFFKLKVKSDISRYKLEFGLCSLEDSKKIHQDAFTLLCEHPDKIEQLPLGFIQNLA
YILSEKYGEKKQVFNMLNSLGKILELQIKEQENMDRKAQITVYLQGIKDYIEK

Vector:p15-TEV-LIC

Growth

Medium:M9 SeMet media kit from Medicilon
Antibiotics:
Procedure:A single colony was inoculated into 10 mL of LB with of ampicillin/chloramphenicol (100 microG/mL and 34 microG/mL respectively) and incubated with shaking at 250 rpm overnight at 37 degC. The culture was transferred into 50 mL of TB with ampicillin/chloramphenicol (100 microG/mL and 34 microG/mL respectively) 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 ampicillin/chloramphenicol (100 microG/mL and 34 microG/mL respectively) and 0.3 mL of antifoam (Sigma) in a 2 L 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 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, both columns were washed with 15 mL binding buffer. The Ni-NTA column was then washed with 200 mL of Wash Buffer at 2 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 2 mM 15 minutes later.

The eluted protein was applied to a Sephadex S200 26/60 gel filtration column (GE Healthcare) pre-equilibrated with gel filtration buffer.The fractions corresponding to the eluted protein peak were collected.

The eluted protein was treated with TEV protease in a molar ratio of protease:protein determined based on measured activity of the available TEV,with the addition of 1 mM tris(2-carboxyethyl) phosphine (TCEP) and 15 mM imidazole, overnight at 4 degC. The cleaved protein was separated from the uncleaved protein by passage through another 2.5 mL Ni-NTA column.

TEV Protease Cleavage: The eluted Cp 14-3-3 protein was treated with TEV protease in a 1:200 molar ratio of protease:protein with the addition of and 15 mM imidazole, overnight at 4degC. The cut protein was separated from the uncut protein by passage through another 2.5 mL Ni-NTA column.The buffer of the cleaved protein was exchanged against the Crystal Buffer and the protein was concentrated to 8 mg/mL using a 15 mL Amicon Ultra centrifugal filter device from Millipore (5 kD cutoff). Aliquots of the purified protein were stored at -80 degC.

Extraction

Procedure
Pellets from 4 L of culture were resuspended to approximately 40 mL/L of cell culture in Binding Bufferwith 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 18000 psi; and the cell lysate was centrifuged using at ~75000 x g for 20 minutes at 10 degC.
Concentration:8 mg/mL
Ligand
MassSpec:
Crystallization: The protein was crystallized by means by hanging drop vapor diffusion in a 24-well Linbro plate. The plate was set with 1 microL cleaved native protein (8 mg/mL) pre-treated with 2 mM DTT and 1 microL buffer in each drop, and 500 microL reservoir volume per well. Cubic crystals grew to maximum size (100 microM) in 15% PEG3350, 0.3 M ammonium acetate and 0.2 M sodium citrate at pH 5.6 at 4 degC after 3 days.
NMR Spectroscopy:
Data Collection:
Data Processing: