Cryptosporidium parvum Adenylate Kinase (CpADK)

PDB:3BE4

Revision

Revision Type:created
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Entry Clone Accession:cgd5_3360
Entry Clone Source:
SGC Clone Accession:cgd5_3360:N4-L219:C4
Tag:mhhhhhhssgrenlyfqg
Host: Ros-Ox

Construct

Prelude:
Sequence:

```
NSKKHNLILIGAPGSGKGTQCEFIKKEYGLAHLSTGDMLREAIKNGTKIGLEAKSIIESGNFVGDEIVLGLVKEKFDLGVCVNGFVL
DGFPRTIPQAEGLAKILSEIGDSLTSVIYFEIDDSEIIERISGRCTHPASGRIYHVKYNPPKQPGIDDVTGEPLVWRDDDNAEAVKV
RLDVFHKQTAPLVKFYEDLGILKRVNAKLPPKEVTEQIKKIL
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Vector:p15-mhl

Growth

Medium:TB
Antibiotics:
Procedure:cgd5-3360 was expressed in E. coli BL21-(DE3)-Rosetta-Oxford cells in Terrific Broth (TB) in the presence of ampicillin/chloramphenicol (50 microg/mL and 25 microg/mL respectively). A single colony was inoculated into 10 mL of LB with of ampicillin/chloramphenicol (50 microg/mL and 25 microg/mL respectively) in a 50 mL Falcon tube and incubated with shaking at 250 rpm overnight at 37 degC. The culture was transferred into 50 mL of TB with 50 microg/mL ampicillin in a 250 mL shaking flask and incubated at 37 degC for 3 hours. Then the culture was transfered into 1.8 L of TB with 50 microg/mL kanamycin and 0.3 mL of antifoam (Sigma) in a 2 L bottle and cultured using the LEX system to an OD 600 of ~5, cooled to 15 degC, and induced with 0.5 mM isopropyl-1-thio-D-galactopyranoside (IPTG) overnight at 15 degC.

Purification

Procedure
STEP1: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 3 mL Ni-NTA (Qiagen) column pre-equilibrated with Binding Buffer at approximately 1 Â□ 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 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 TCEP was added to 0.5 mM after approximately 15 more minutes.

Step 2, Cutting His Tag: TEV enzyme(4 mg/ml with activity of 1 to 20) added to the protein and dialysis in 10 mM HEPES, pH 7.5, 500 mM NaCl, 5mM DTT overnight, the day after the protein passed through Ni_NTA column(2ml) and washed with Binding buffer, then was concentarted using a 15 mL Amicon Ultra centrifugal filter device (Millipore). TECEP (5mM) was added to the concentrated protein. The protein sample identity were evalulated by mass spectroscopy.

STEP3:The sample was loaded onto a Sephadex S75 26/60 gel filtration column pre-equilibrated with 10 mM HEPES, pH 7.5 and 500 mM NaCl. The collected fractions corresponding to the correct eluted protein peak were concentrated using a 15 mL Amicon Ultra centrifugal filter device (Millipore). TECEP (5mM) and MgCl2 (5mM) was added to the concentrated protein. The protein sample identity were evalulated by mass spectroscopy. The concentrated sample (62 mg/ml) was stored at 4 degC.

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 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 a Beckman JA-25.50 rotor at ~75000 x g (24000 rpms) for 20 minutes at 10 degC.
Concentration:
Ligand
10mM Adenosine5pentaphosphate pentalithium salt added to the protein before setting up the plate**MassSpec:**
Crystallization:MAY5RU, well E1, drop 125% glycerolThe protein was crystallized at 4 degC in 2.5M NH4SO4, 0.1M BTP PH 7 using the sitting drop vapor diffusion method.
NMR Spectroscopy:
Data Collection:
Data Processing: