CTPS2

PDB:3IHL

Revision

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Entry Clone Accession:gi|23271202
Entry Clone Source: Mammalian Gene Collection (RZPD)
SGC Clone Accession:CTPS2A-k061
Tag:C-terminal hexahistidine tag *ahhhhhh
Host:*E.coli* BL21(DE3) R3 pRARE, where R3 denotes a derivative of BL21(DE3) resistant to a strain of T1 bacteriophage (SGC Oxford) and the pRARE plasmid originating from the Rosetta strain (Novagen) supplies tRNAs for rare codons.

Construct

Prelude:
Sequence:

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MKYILVTGGVISGIGKGIIASSIGTILKSCGLRVTAIKIDPYINIDAGTFSPYEHGEVFVLNDGGEVDLDLGNYERFLDINLYKDNN
ITTGKIYQHVINKERRGDYLGKTVQVVPHITDAVQEWVMNQAKVPVDGNKEEPQICVIELGGTIGDIEGMPFVEAFRQFQFKAKREN
FCNIHVSLVPQLSATGEQKTKPTQNSVRALRGLGLSPDLIVCRSSTPIEMAVKEKISMFCHVNPEQVICIHDVSSTYRVPVLLEEQS
IVKYFKERLHLPIG*ahhhhhh
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Vector:pNIC-CH2

Growth

Medium:
Antibiotics:
Procedure: Cells from a glycerol stock were grown in 20 ml TB supplemented with 8 g/l glycerol, 100 µg/ml kanamycin and 34 µg/ml chloramphenicol at 30 °C overnight. The overnight culture (20 ml) was used to inoculate 1.5 l TB supplemented with 8 g/l glycerol, 50 µg/ml kanamycin and approximately 200 µl 204 Antifoam A6426 (Sigma). The culture was grown in a LEX bioreactor system (Harbinger Biotechnology) at 37 °C for four hours prior to induction without monitoring OD600. Prior to induction the culture was down-tempered to 18 °C over a period of 1 hour before target expression was induced by addition of 0.5 mM IPTG. Expression was allowed to continue overnight and cells were harvested the following morning by centrifugation (4,400 x *g*, 10 min, 4 °C). The resulting cell pellet (18 g wet cell weight) was resuspended in lysis buffer (1.5 ml/g cell pellet), supplemented with 2000 U Benzonase (Merck) and one tablet of Complete EDTA-free protease inhibitor (Roche Applied Science). The cell suspension was stored at -80 °C.

Purification

Procedure
Columns
IMAC: Ni-charged 1 ml HiTrap Chelating HP (GE Healthcare)
Gel filtration column: HiLoad 16/60 Superdex 200 Prep Grade (GE Healthcare)
Procedure
Purification of the protein was performed as a two step process on an ÄKTAxpress system (GE Healthcare). Prior to purification, columns were equilibrated with IMAC wash1 buffer and gel filtration buffer, respectively. The filtered lysate was loaded onto the Ni-charged HiTrap Chelating column and washed with IMAC wash1 buffer followed by IMAC wash2 buffer. Bound protein was eluted from the IMAC column with IMAC elution buffer and automatically loaded onto the gel filtration column. Fractions containing the target protein were pooled and fresh TCEP was added to a final concentration of 2 mM. The protein was subsequently concentrated using an Amicon Ultra-15 centrifugal filter device, 10,000 NMWL (Millipore) to 16.9 mg/ml in a volume of 0.7 ml. The mass of the protein used for crystallization was determined by mass spectrometry to 31 484 Da, which is sligthly higher than the calculated mass 31 441 Da.

Extraction

Procedure
The cell suspension was completely thawed in warm water. Cells were disrupted by sonication (Vibra-Cell, Sonics) at 80% amplitude for 3 min effective time (pulsed 4s on, 4s off) and cell debris was removed by centrifugation (49,000 x *g*, 20 min, 4 ºC). The supernatant was decanted and filtered through a 0.45 µm flask filter.
Concentration:
Ligand
MassSpec:
Crystallization: Crystals were obtained by the sitting drop vapour diffusion method in a 96-well plate. 0.1 µl protein solution (16.9 mg/ml) containing 5mM ATP and 5mM MgCl2, was mixed with 0.1 µl of well solution consisting of 0.8 M Succinic Acid. The plate was incubated at 4 ºC and crystals appeared within 5 days. The crystals were quickly transferred to a cryo solution consisting of 0.8M Succinic Acid, 0.3M NaCl and 23% Glycerol, and flash frozen in liquid nitrogen.
NMR Spectroscopy:
Data Collection: Diffraction data to 2.8 Å resolution was collected at ESRF, beamline ID23-1.
Data Processing: The structure was solved by molecular replacement using PHASER and our previously determined structure of CTPS as search model (PDB: 2VO1). The space group was P62 2 2 (180) with cell dimensions a=168.25 Å b=168.25 Å c=132.00 Å. Two monomers were located in the asymmetric unit and the PISA server predicts this CTPS2 structure to be tetrameric, however the protein was running as a dimer on the gelfiltration column. Despite being co-crystallized in the presence of ATP the structure contains ADP andREFMAC5 was used for refinement and Coot for model building. Data in the interval 50-2.80 Å resolution was used and at the end of the refinement the R values were: R=21.4% and Rfree=27.0%. Coordinates for the crystal structure were deposited in the Protein Data Bank, accession code 3IHL.